Autologous Skin Cell Suspension For Accelerating Re-Epithelization Of Split Thickness Donor Sites

by

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LIST OF ABBREVATIONS

ECM	Extra cellular matrix
STSG	Split thickness skin grafting
NS	Normal saline
PDGF	Platelet derived growth factor
SD	Standard deviation
SAM	Synthetic adhesive moisture
EGF	Epidermal growth factor

ABSTRACT

AIMS & OBJECTIVES

To evaluate the safety and efficacy of local injection of autologous skin cell suspension in donor site of split skin thickness graft with colloid dressing.

MATERIALS AND METHODS-

A total of 84 patients were included the study and were randomly divided into two groups ,42 patients in skin cell suspension injection into donor site group and 42 patients in colloid dressing group. Age between 18-80 years, injection of skin suspension cell into the donor site and dressing opened on 3rd day after that every 3 weeks follow up.

RESULTS- in our study, we found that the mean healing time in the cases was 13 days, whereas in control group, it was found to be 17 days, P-value was very significant P value 0.001 which states that healing time was faster in cases compared to control group.

CONCLUSION- In our study, there was no statistical difference in distribution of patients between the study group and control group with respect to age and sex.

whereas skin cell suspension injecting group showed faster healing when compared to hydrocolloid dressings with minimal complications.

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INTRODUCTION

It is well accepted fact that largest organ in human body is skin. It corresponds to about 16% of entire body weight. It acts as a protective barrier and has a major role in regulation of body temperature also its other significant roles are metabolic particularly with respect to protein and vitamin D It has been proven that the epidermal layer of skin manufactures maximum quantity of vitamin D[1].

Other than its protective functions it also a role in immunity and has antigenic functions that is important with respect to allotransplantation[2]. In order to restore the protective barrier which is of utmost significance can be performed by various methods for example grafting

Skin grafting is performed for a lot of reasons like faster wound healing to prevent loss of proteins and fluid loss, prevent secondary infection, to decrease rate of contracture and for cosmetic purpose. Before doing grafting, patients should be explained clearly that due to exposed free nerve endings donor site will be painful and cause more discomfort than graft site. Although there are many new updates still split thickness skin grafting remains procedure of choice in most plastic surgery. The procedure of skin grafting is most almost similar but treating the donor site is challenge and still debatable. The donor site tends to receive less attention than graft site and often heals slow causing pain and uneasiness to the patient. It is very common seen that patients have more pain at the donor site as compared the graft site.

It has already been mentioned that skin is protective barrier against pathogens escape from extracellular fluid so following split thickness skin grafting there is loss of blood as well as proteins and fluid from donor site finally this protein rich exudate and blood forms a coagulum which leads to scab formation that helps in formation of

epithelium and hastens wound healing but this scab might also cause superadded infection of epithelium at donor site which can lead to loss of full thickness of skin.

All the donor sites there occurs in regeneration of epidermis from left over epithelium and also from the left-out hair follicle sebaceous and sweat glands. This corresponds to first stage in healing. this is followed by cellular migration outside till wound is epithelized again. It has been seen normally that full reepithelization takes 10-14days, which also may be variable since it depends on the graft thickness[3]. Also rate of healing is dependent on age and nutritional status of the patient. In elderly, healing may be faster if a small quantity of graft is used and then spread widely Over the raw area with wider fenestrations. Spread of the graft over the wound and reepithelization at the donor site occurs simultaneously.

Following this generate moderate to high amounts of exudate for 3-4 days which however depends on the size of wound as soon as exudates reduces, the process of reepithelization hastens. a proper wound dressing is needed to reduce patients' pain and uneasiness. Although while removal it may lead to delayed healing and severe pain to patient. It is essential to keep the dressing at donor area until the wound has healed. Although it can be unlikely because of wound site skin texture, patient variability etc., while managing hasten the healing process, reducing the rates of newer complications and managing pain scale of the patient.

NEED FOR THE STUDY: Split thickness skin graft remains the most frequently used reconstructive option for skin and soft tissue defects

- The procedure involves harvesting of the full epidermis and Split thickness skin graft remains the most frequently used part of dermis but creates a secondary wound at the donor site.

- Patient may experience donor site discomfort (pain and itching), delayed healing and infection, an unsatisfactory cosmetic appearance and reduced quality of life.
- Co-morbidities such as ageing, poor nutrition, immobility, smoking, diabetes and peripheral vascular disease contribute to impaired donor-site healing[4].
- Epidermal substitute is an autograft derived from split thickness grafting or from cell line bioreactor expansion. Epidermal replacements are created by expansion of patient derived cells in the laboratory until enough cell mass is generated to be transferred to the wound[5].
- Autologous, non-cultured, heterogenous skin cell suspension which can be obtained which includes 65% keratinocytes 30% fibroblasts and 3-5 % melanocytes. With only a small donor population of autologous basal layer cells, cells of suspension contain viable melanocytes, it has been used for pigmentation. Current therapeutic strategies for STSG donor sites are focused on creating an optimal environment that allows rapid re-epithelization by accelerating keratinocyte proliferation[6].

AIM AND OBJECTIVE OF THE STUDY

To evaluate the safety and efficacy of local injection of autologous skin cell suspension in donor site of spilt skin thickness graft with colloid dressing.

RESEARCH HYPOTHESIS

Skin cell suspension is a safe, simple, biocompatible technique as a definitive management of donor site injection in split thickness skin grafting without any adverse events.

REVIEW OF LITERATURE

The skin grafting history begins in 3000-2500BC in India in Sanskrit texts it is documented skin transplants done by Hindus. It has been mentioned that for adultery and theft mutilation was the punishment which was reconstructed by potters and tile makers of the koomas caste. Previously graft was taken by slapping with wooden paddle until red and congested then cut with a leaf to the appropriate size, graft place usually from buttock, thigh[7].

For advance in skin transplantation it took many attempts in plastic and reconstructive surgery. Brancas in Italy in 1442 did skin grafting he used new technique skin of slaves transplanted to his masters. for his work he didn't get recognition instead of him country man appreciated. After one century later pioneer modern plastic surgeon Tagliacozzi recognised the work of Brancas and published the method of skin grafting. He also involved in reconstruction of damaged nose due to syphilis and also war field facial wound repair. Previously it was like business work, later it transformed to research work and Tagliacozzi in 1597 published his work in 'Decurtorumchirugia per insitionem'[7].

In 1663 The Royal society of London skin grafting experiment was done on a dog. After many attempts later they left on animals because research on animals was temporarily banned. Garengeot in 1731 his experiment was not successful due to wine was warming and then reattaching a military person's nose which was partially amputated. Italians were also involved in skin grafting work. In Europe in 1794 was first time started skin grafting, before that in India concept of skin grafting was started. Skin grafting acceptance and advancement was dramatically improved in 19th century. Baronio[8] first successful autograft was done in 1804 by using autograft of

backs of sheep. First successful autograft of human in 1823 by Bunger[7]. many attempts were done in India for skin grafting which was done in rhinoplasty. Bunger full thickness skin grafting was taken from thigh and used for nasal defects.

First split thickness skin grafting was done by Swiss surgeon Reverdin[7] allograft was very thin [epidermis] pieces of epidermis, he also demonstrated wound heals rapidly after skin grafting. After few years Olilier, in split thickness skin grafting he used both part of dermis and full epidermis as a content, which gives better and faster wound healing with minimal scarring. Pollock used his idea in skin grafting in bums in 1871 he used small pieces of his own hand to burn victims, he used that skin to large area of burn wounds.

With his great idea of autologous split thickness skin grafting treated many burn patients, it leads to modern ideas. Later in 19th century Wolfe used full thickness skin grafting for eyelids to treat ectropion which was clinically acceptable. First skin grafting to human acceptable taken from cadaveric skin by Girdner. Skin grafting reduced mortality in burn patients and care has been improved, 19th century practice improved.

Understanding of the biology of wound healing and immunology of transplant rejection was low and improved quality of life in skin grafting. In 1960, George Winter concluded that wound re epithelization faster in a moist environment he referred as a pioneer worker in wound care management[9]. Mainly concentered on graft site donor site heals by epithelization process and wound is covered with petrolatum [paraffin] gauze. and to dry out.

Skin grafting donor site drying was done by using hair dryer's air drying, and heat blankets by this method leads to pain and uneasiness and regular watching was

needed trim the edges of dressing should be done to shed from healing wound. open dressing touches the cloths of the patient and its cause pain, irritation. Repeated dressing process causes trauma and causes the wound at donor site open dressing which is healed to scab which is not recommended in evidenced practice compared to moist dressing.

In recent years, much has been published highlighting the benefits of moisture-retentive dressings in treating donor sites. Moisture-retentive dressings that have been used include hydrocolloids, foams, and transparent thin film dressings, alone or in combination with absorbent materials such as alginates, hydrofibers or gauze. While hydrocolloids and foams provide the needed absorbency, they must be removed whenever wound inspection is required, increasing treatment cost and the risk of traumatizing the wound.

Thin film dressings allow for wound visualization, but usually fail to contain the drainage for more than 24 hours, even when used secondary to other absorbent dressings (which also negates the benefit of transparency). Number of newer methods of skin grafting at donor sites dressing has been shown rapid healing and re epithelization[1]. In large area of burns reharvesting can be done in split thickness skin grafting which improves healing process epithelization and less scarring.

Sometimes due to infection of donor sites partial thickness skin graft area turns into full thickness skin graft area due to erosion and delaying in healing[2,10]. Healing of donor site depends on the many factors like size, site selection, skin preparation, graft depth, hemostasis, choice of dressings and infections[3, 11]. The patient health will be good in quality wise if skin graft donor site heals in faster rate. Next time we can use same area for re harvesting of skin if it has completely re epithelized. For producing

good and viable split thickness from previously harvested area takes as long as 3weeks4. Once a graft is harvested the dermis lost at donor site is not replaced. Only reepithelization occurs and epidermis is formed. The dermis harvested is a net loss at the donor site. Repeated harvesting at same donor site cause progressive thinning of dermis at donor site. Subsequent layers of graft are less elastic. Dehydration and infection can be prevented by an ideal dressing at donor sites. conventional dressing, we are using are paraffin gauze and gauze dressing. Use of paraffin gauze dressing has low acceptance to donor sites due permeable to infection and its dry very fast, it may slip easily. It will be on the nerve dings; it may form bulk it may cause pain. irritation to patient with this this dressing and can't take bath.

ANATOMY

ANATOMY OF THE SKIN

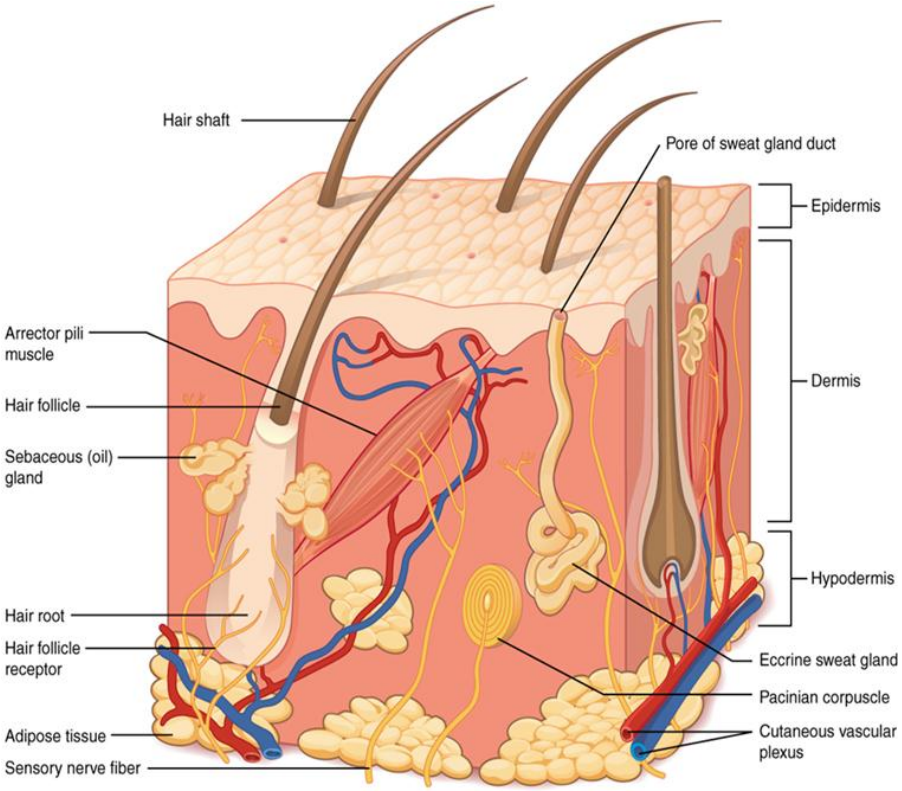


Figure 1

The skin has two layers; epidermis / outer layer is derived from ectoderm[12]. Dermis is derived from mesoderm its thicker layer. 5% of skin constitutes the epidermis and dermis is the remaining 95 percent. The thickness of the skin varies according to the site, gender and age the thickest skin is of the soles and palms, eyelids and in post auricular region are thinnest skin. Compare to females, males are having thicker skin in all anatomic location. In children skin is thin till 11 years gradually thickens as age progresses and skin thickness continues till 4th or 5th decade, after 5th decade skin gradually progresses to thinning due loss of epithelial appendages ground substance and loss of dermal elastic fiber[13].

EPIDERMIS

Superficial layer of skin is epidermis, and is formed by keratinocytes which are undergoing terminal maturation arranged in layers. Cornification is defined as a procedure which involves the migration of these keratinized cells to the surface. The other cells of the epidermis with no keratin are[14]:

LANGERHANS CELLS – These are the antigen presenting dendritic cells

MELANOCYTES-These are responsible for the formation of the pigment melanin.

MERKEL CELLS – sensory mechanoreceptors.

LAYERS OF THE EPIDERMIS

The epidermis is formed into 5 layers (strata) of keratinocytes – the layers forming from inside to outside are-

STRATUM BASALE – This is the deepest layer, in which many cells are active and dividing. The basal stratum is a single cell layer consisting mainly of basal cells.

Basal cells are the cells which are cuboidal in shape which are basically stem cells. The next layer which is the dermis and the Stratum Basale are separated by a basement membrane, it is composed of collagen and proteins. Two other kinds of cells are discovered in the basal stratum Basale spread among the basal cells. Merkel cell is one of these which acts as receptor for sensory stimulus. The second is a melanocyte, a pigment melanin-producing cell. This melanin is the basic pigment which is responsible for the colour of skin and the hair[16].

STRATUM SPINOSUM– keratinocytes are joined by tight intercellular junctions called desmosomes. This layer contains cells that change shape from columnar to polygonal. The desmosomes interlock and reinforce the bond between the cells. It is important to note that this layer's "spiny" nature is a staining process artefact. The stratum spinosum consists of 8 to 10 layers of keratinocytes created in the Stratum Basale as a consequence of cell division. A sort of dendritic cell called the Langerhans cell is interspersed among the keratinocytes of this layer, which have the phagocytic function of engulfing foreign materials. The keratinocytes cause keratin synthesis; repelling glycolipid that avoid water loss from body, making the skin comparatively water-proof. The cells (three to five layers deep) become flatter, their cell membranes thicken, generating huge quantities of fibrous protein keratin, and keratohyalin, accumulating in the cells as lamellar granules[17].

STRATUM LUCIDUM– The cells which are present lose nuclei and cause increased keratin production. These cells are tightly bound with eleiden, a clear, lipid-rich protein obtained from keratohyalin that gives lucid texture[18].

STRATUM CORNEUM – The epidermis ' main layer. In this layer the cells are bound tightly to each other and hence our skin is waterproof. This layer is essential to

prevent the entry of the foreign particles such as bacteria, and bugs. Cells lose all organs, keep producing keratin

Typically, a keratinocyte requires a minimum of 30-40 days to move from the basal layer to the surface. Over a period of about 4 weeks, the entire layer is substituted. Cosmetic procedures, help to remove upper layer and maintain the skin texture which appears “fresh" and healthy in appearance[19].

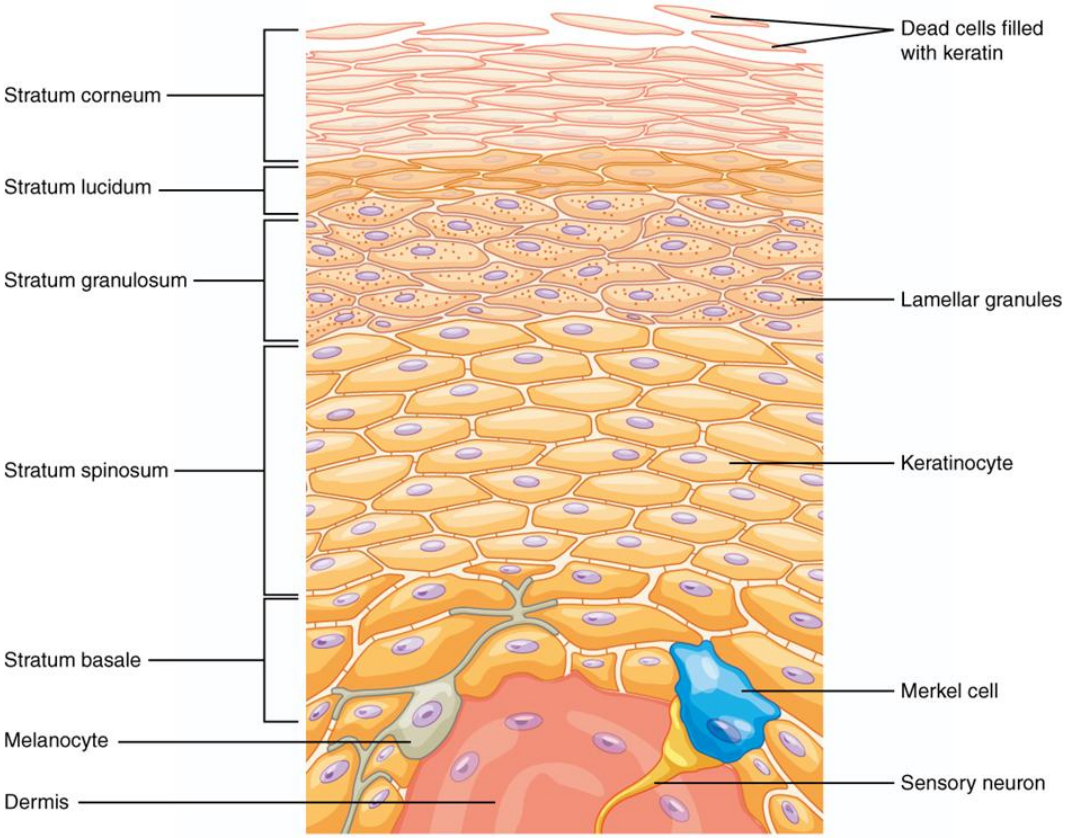


Figure 2

LAYERS OF EPIDERMIS

DERMIS

The dermis could be regarded as the "core" of the integumentary structure (derma-= "skin"), as separate from the epidermis (epi-= "on" or "over") and hypodermis (hypo-

= "below"). It includes vessels of blood and lymph, nerves, and other structures such as hair follicles and glands of sweat. The dermis is composed of 2 layers, the deeper reticular dermis and the superficial papillary dermis which are complex in nature. The papillary dermis is composed of connective tissue which has capillaries, collagen, reticular fibers This superficial layer goes into the epidermis to form finger-like dermal papillae. Fibroblast, a tiny amount of fat cells (adipocytes) and an abundance of tiny blood vessels are present in the papillary layer. This layer also contains nerve fibers, touch receptors, capillaries the touch receptors are described as Meissner corpuscles[16]. The reticular dermis is thick layer of connective tissue which contain blood vessels, elastic fibers, and collagen fibers This layer is vascularized with sensory and sympathetic nerves innervation. Due to a tight mesh of fibers, the reticular layer appears reticulated (net-like). Elastin fibers provide skin with some elasticity, enabling motion. The tensile strength is provided by the collagen fibers. The reticular layer contains fibroblasts, lymphatics, epidermal appendages, mast cells and nerve endings. The mucopolysaccharides (hyaluronic acid and chondroitin sulfates) and glycoproteins surrounds the dermis parts.

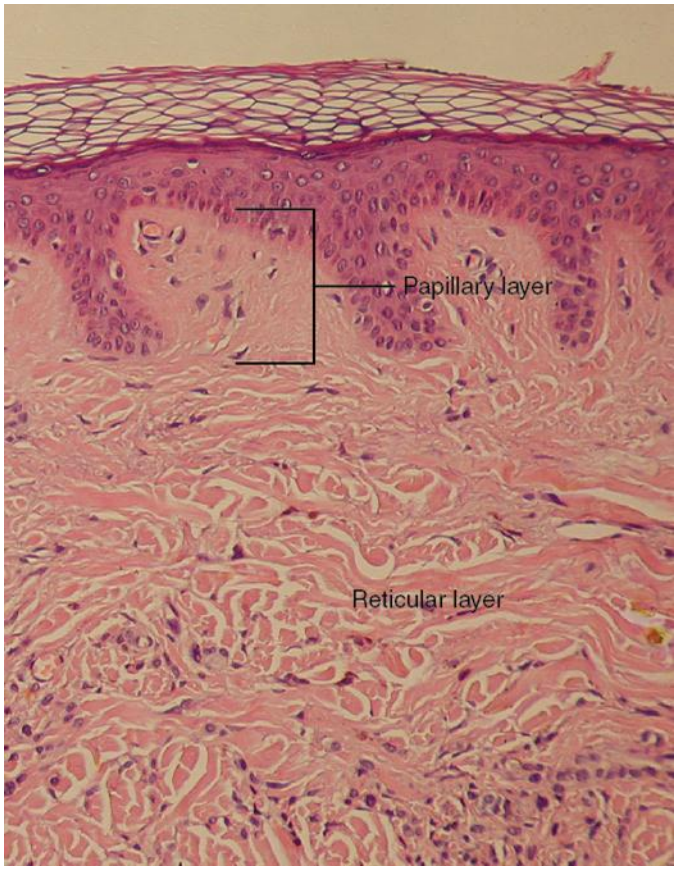


Figure 3

HYPODERMIS

The hypodermis is a layer below the dermis which connects the skin to the bones and muscles. The hypodermis is a well-vascularized, connective tissue and adipose tissue that acts as a fat storage and offers the integument with insulation and cushioning. In the hypodermis, adipose tissue comprises of cells called adipocytes. This acts as an energy reserve, avoid heat loss, and helps to avoid trauma from underlying structures. The fat deposits and accumulates depending on hormones (testosterone, estrogen, insulin, glucagon, leptin and others) and genetic variables within the hypodermis[18].

PIGMENTATION

A number of pigments, including carotene, Melanin, hemoglobin, influence the skin colour. That melanin is generated by melanocyte cells that are found to be scattered throughout the epidermis basal stratum. The melanin is transmitted through a cellular vesicle called a melanosome into the keratinocytes. Melanin takes place in two main types. Eumelanin is black and brown, while pheomelanin is red. Dark-skinned people generate more melanin than pale-skinned people. Keratinocyte accumulation of melanin outcomes in skin darkening, or a tan[20].

SOURCES OF EPITHELIAL CELLS

Epidermal appendages are sources rich of epithelial cells which re-epithelialize in individuals with partial surface burns when the structure covering this is destroyed. Skin graft harvesting abrasions or split-thickness. These intradermal epithelial buildings are lined with epithelial cells with the capacity for division and differentiation, such as sebaceous glands, sweat glands and hair follicles. They are found deep inside the dermis and deep inside the dermis in the subcutaneous fat[18].

SEBACEOUS GLANDS

Sebaceous glands, secrete sebum that lubricates the skin and makes it moisturizing. They are present all throughout the body's surface except the palms, soles, and dorsum. They are biggest and most focused in the face and scalp where acne originates[21].

THE SWEAT GLANDS

They are found throughout the body's surface except the feet's palms, soles, and dorsum. They are the biggest and most concentrating in the face and scalp where acne

originates. The glands ' major role is to generate the sweat that cools the body by evaporating[22].

THE APOCRINE GLANDS

In structure, apocrine glands are comparable but they are not the same as eccrine sweat glands. They are maximum in the axillae and anogenital areas, they are likely to act as vestigial sexual function as they generate odour and function only after puberty[22].

HAIR FOLLICLES

Important source of epithelial cells is the hair follicle, and many of these epidermal appendages open in the hair follicle instead of directly on the skin surface[23].

WOUND HEALING

Wound healing is a complicated method in which the skin is repaired after injury and the tissues under it. When the barrier is breached, the damage is repaired by a controlled series of biochemical occurrences.

This process is classified into the 4 stages. Blood clotting may be regarded part of the inflammation stage.

STAGES IN WOUND HEALING

HEMOSTASIS

Platelets in the blood adhere to the wounded site within the few minutes of injury. This mechanism activates the platelets and causes following. They alter to amorphous form, more appropriate for coagulation, and to encourage coagulation, they cause release of chemical signals which results in fibrin activation, which forms

a mesh and acts as a "glue" for binding platelets together. This causes a clot that slows down / prevents further bleeding in the blood vessel.

INFLAMMATION

Damaged cells with bacteria and other pathogens or debris, are removed during this stage. This occurs through the phagocytosis process where white blood cells "eat" debris by swallowing it up. Platelet-derived growth factors that trigger migration and cell division during the proliferative phase are released into the wound[23].

PROLIFERATION

Angiogenesis, deposition of collagen, formation of granulation tissue, epithelialization, and contraction of wounds occur. Vascular endothelial cells create fresh blood vessels in angiogenesis. By excreting collagen and fibronectin, fibroblasts expand and form a fresh, temporary extracellular matrix (ECM) in fibroplasia and granulation tissue formation. Re-epithelialization causes the cells to' crawl ' over the wound bed offering cover for the fresh tissue. In wound contraction, myofibroblasts reduce the wound size by gripping the wound

edges and tightening similar to that found in smooth muscle cells. When the roles of the cells are close to completion, there is apoptosis of unneeded cells[23, 24].

MATURATION

Collagen is realigned along tension lines during maturation and remodeling, and cells no

longer required are removed through programmed cell death or apoptosis[25,26,27].

SKIN GRAFTS

Thought to have originated in India more than 2,500 years ago, skin grafting is the next step on the reconstructive ladder for the closure of a wound that cannot be closed primarily. Skin transplanted from one location to another on the same individual is termed an autogenous graft or autograft. Skin grafts are classified as either split-thickness or full-thickness, depending on the amount of dermis included in the graft. A partial or split-thickness skin graft (STSG) contains a variable thickness of dermis, while a full thickness skin graft (FTSG) contains the entire dermis. Split-thickness skin grafts are further categorized as thin (0.005-0.012 inch), intermediate (0.012-0.018 inch), or thick (0.018-0.030 inch) based on the thickness of graft harvested.

The thicker the dermal component, the more the characteristics of normal skin are maintained following grafting. This is because of the greater collagen content and the larger number of dermal vascular plexuses and epithelial appendages contained within thicker grafts[28].

However, thicker grafts require more favourable conditions for survival because of the greater amount of tissue requiring revascularization. The choice between full- and split-thickness skin grafting depends on wound condition, location, and size, as well as aesthetic considerations.

FULL-THICKNESS SKIN GRAFTS

Full-thickness skin grafts are ideal for visible areas of the face that are inaccessible to local flaps or when local flaps are not indicated. Full thickness grafts retain more of the characteristics of normal skin, including colour, texture, and thickness, when

compared with split thickness grafts. Full-thickness grafts also undergo less contraction while

healing. This is important on the face as well as on the hands and over mobile joint surfaces. Full-thickness grafts in children are more likely to grow with the individual. However, full-thickness skin grafts are limited to relatively small, uncontaminated, well-vascularized wounds and thus do not have as wide a range of application as split-thickness grafts.

Donor sites must be closed primarily or, more rarely, resurfaced with a split-thickness graft from another site[29].

SPLIT-THICKNESS SKIN GRAFTS

Split-thickness skin grafts can tolerate fewer ideal conditions for survival and have a much broader range of application. They are used to resurface large wounds, line cavities, resurface mucosal deficits, close donor sites of flaps, and resurface muscle flaps. They also are used to achieve temporary closure of wounds created by the removal of lesions that require pathologic examination prior to definitive reconstruction. Split-thickness skin graft donor sites heal spontaneously with cells supplied by the remaining epidermal appendages, and these donor sites may be harvested once healing is complete. Split-thickness grafts also have significant disadvantages that must be considered[30]. Split-thickness grafts are more fragile, especially when placed over areas with little underlying soft tissue bulk for support, and usually cannot withstand subsequent radiation therapy. They contract more during healing, do not grow with the individual, and tend to be smoother and shinier than normal skin because of the absence of skin appendages in the graft. They tend to be abnormally pigmented, either pale or white, or alternatively, hyper pigmented,

particularly in darker-skinned individuals. Their lack of thickness, abnormally smooth texture, lack of hair growth, and abnormal pigmentation make these grafts more functional than cosmetic. When used to resurface large burns of the face, split-thickness grafts may produce an undesirable mask like appearance.

Finally, the wound created at the donor site from which the graft is harvested is often more painful than the recipient site to which the graft is applied.

GRAFT SURVIVAL AND HEALING

The ultimate success of a skin graft, or its "take," depends on nutrient uptake and vascular ingrowth from the recipient bed, which occurs in 3 phases. The first phase takes place during the first 24-48hours. The graft is initially bound to the recipient site through formation of a fibrin layer and undergoes diffusion of nutrients by capillary action from the recipient bed by process called ‘plasmatic imbibition’[29, 31].

The second phase involves the process of inosculation, in which the donor and recipient end capillaries are aligned and establish a vascular network.

Revascularization of the graft is accomplished through those capillaries as well as by in growth of new vessels through neovascularization in the third and final phase, which is generally complete within 4-7 days. Reinnervation of skin grafts begins approximately 2-4 weeks after grafting and occurs by ingrowth of nerve fibers from the recipient bed and surrounding tissue. Sensory return is greater in full-thickness grafts because they contain a higher content of neurilemmal sheaths. Similarly, hair follicles may be transferred with a full-thickness graft, which allows the graft to demonstrate the hair growth of the donor site. Split-thickness grafts are usually hairless. The amount of dermis present in the graft determines the degree of

contraction immediately after harvest from the donor site and following placement and revascularization in the recipient bed. Freshly harvested grafts undergo immediate recoil as a result of elastin in the dermis in a phenomenon termed primary contraction. Therefore, a full-thickness skin graft contracts more initially following harvest as it contains the dermis in its entirety. Secondary contraction is likely due to myofibroblast activity in the wound bed and is defined as the contraction of a healed graft. The degree of secondary contraction is inversely related to the thickness of the skin graft[32].

Accordingly, split-thickness skin grafts contract more than full thickness grafts following placement in the recipient bed. For that reason, full-thickness grafts are preferably used in areas that would be significantly impacted functionally or aesthetically by scarring or scar contracture, such as the head and neck, hands, genitals, or breast. Current investigations into methods to reduce initial contraction and subsequent need for contracture release include early mechanical restraint immediately following grafting as well as application of topical agents to delay keratinocyte differentiation or prevent crosslink formation.

DONOR SITE SELECTION

Donor site selection is based on multiple factors, including skin colour, texture, dermal thickness, vascularity, and anticipated donor site. Split-thickness skin grafts are commonly harvested from the thigh, buttocks, abdominal wall, or scalp. The method of harvesting the split-thickness skin graft depends primarily on the size and thickness needed for coverage of the defect. Smaller grafts can be taken using a "pinch graft" technique using a scalpel blade; slightly larger freehand grafts can be obtained with a Weck blade. Powered dermatomes such as the Zimmer (Zimmer,

Inc.,) are most commonly used to harvest split-thickness skin grafts, as they have a rapidly oscillating blade that can be set at an adjustable depth and width for appropriate coverage of the defect[31].

Lidocaine with epinephrine may be injected subcutaneously at the donor site prior to harvesting, which aids in reducing blood loss and providing greater tissue turgor to facilitate graft harvest.

The planned harvest site and dermatome can be lubricated with mineral oil, sterile saline, or Shur-Clens (ConvaTec, Princeton, NJ) to enable easy gliding of the dermatome over the skin. Epinephrine-soaked gauze may be applied to the donor site immediately following harvest to achieve haemostasis.

HAEMOSTASIS

Bleeding from a donor site is similar in amount to that of tangential excision of a fresh, deep dermal burn, i.e., diffuse, puncture, and profuse. Bleeding from a reused donor is even more profuse and again an analogy can be made with a tangential excision of a hyperemic wound. Because blood loss will be substantial, hemostasis at the donor site should be controlled before pursuing wound excision[31].

The ideal situation is the use of two teams, one whose role is to obtain skin grafts and maintain hemostasis. Pressure followed by application of fine mesh gauze or xeroform gauze, again followed by pressure (1 to 2 minutes) is usually adequate to control bleeding. As with the excised wound, topical thrombin or a diluted epinephrine solution can also be used.

DONOR SITE HEALING

The split-thickness skin graft donor site epidermis regenerates by secondary epithelialization from the wound edges and from immigration of dermal cells originating in the shafts of hair follicles as well as adnexal structures remaining in the dermis. Although the dermis never regenerates, the same site may be harvested again for subsequent grafts because only a portion is removed in a split-thickness graft. A skin graft is typically a thickness of skin comparable in depth to a partial thickness skin loss, i.e., epidermis and the upper third of the dermis. Typically, the slice of skin is 0.001 to 0.014 inches thick.

A split thickness skin graft (STSG) of 0.001 inches typically contains the epidermis and upper third of the dermis, i.e., the papillary dermis. Appendages in the dermis to allow re- epithelialization in about 14 days. A 0.15-inch thickness graft usually contains about half of the dermal layer (or more) which includes a portion of the papillary dermis. Fewer epidermal cells remain and the site heals much slower, similar to a mid to deep dermal burn. Protection of remaining epidermal and dermal elements is essential to allow for proper healing. The most bioactive portion of the dermis is removed with a STSG, i.e., the papillary dermis. The donor site healing will depend on when bioactive dermal growth enhancing factors are produced on the surface which can then stimulate re-epithelialization. Placement of a tissue engineered wound matrix on the donor site will provide active extracellular matrix components to stimulate healing. The usual time for re-epithelialization of a donor site of 0.010 inch in depth, is about 14 days in a patient 10-50 years old and about 21 days in a toddler or geriatric patient using a typical grease gauze dressing[31].

The donor site is not without impaired cosmesis, however, as (1) hypertrophic scar formation, (2) Thin Unstable scar or (3) changes in skin pigmentation can occur upon healing. In the first 3–4 days' post-surgery, the donor site wound produces moderate to heavy amounts of exudate, depending on the size of the wound area. After this, exudate levels diminish as re-epithelialization progresses[29,31].

The healing of donor site wounds can be divided into two phases.

The 'wet phase' is when copious amounts of exudate are produced. An absorbent dressing such as a foam, alginate or hydro-braine dressing can be used to absorb the excess.

The 'dry phase' is when the exudate levels fall dramatically and the wound bed becomes dry. It can be treated with a simple non-adherent silicone dressing, which can remain undisturbed without adhering to the wound bed for several days or until the wound has healed. It is in the

patient's best interests that one dressing is applied and remains in situ until healing is achieved. Unfortunately, if an alginate or hydro-broine dressing is left in situ throughout healing, the dressing is likely to dry out and possibly adhere to the wound bed[32]. Foam dressings draw excess moisture away and have low adherence to the wound bed so may be appropriate (Wilkinson,1997). Perhaps the most appropriate dressing is a simple no adherent silicone dressing (Platt et al, 1996). During the initial 'wet phase' this would need padding and the outer dressing renewed regularly, otherwise the weight of the dressing could cause slippage, resulting in exposure of the wound and distress to the patient.

COMPLICATIONS

A number of complications can occur in the donor site. Infection can occur which can result in deepening and possibly conversion of the wound to full thickness loss and ulceration.

Infection is usually evident from surrounding cellulitis. Systematic antibiotics as well as topical antibiotics are required for treatment. Blistering and continued breakdown are also seen, especially with deep donors or donors in small children or the elderly. Healing usually occurs in time. Hyper or hypo-pigmentation may persist for long periods of time and may be permanent. Hypertrophic scarring is seen especially in dark-skinned persons and with deep donor sites.

Delayed healing of skin donor sites may be costly and life threatening, especially in patients with large body-surface area burns. A donor site dressing should maximize the ability of the wound to heal without increasing the risk of local infection, systemic infection, or both. Specifically, the possibility of a secondary infection may either slow the healing process or ultimately convert the donor site to a full thickness wound. A number of materials, ranging from gauze to biological agents, have been investigated for use as donor site dressings[33].

DONOR SITE HEALING AND THE WOUND ENVIRONMENT

Normal wound healing is a series of orchestrated events with an initiation phase, collagen deposition phase, keratinocyte ingrowth phase, and maturation phase. The process is dependent on oxygen delivery to tissue, pH of tissue, and development of a local wound environment conducive to the cells involved in repair. Growth factors

provided exogenously or by repairing cells have been the focal point of numerous wound healing investigations[34, 35].

Brown and associates investigated epiderma1 growth factor (EGF) in association with skin graft donor site healing.

This work showed that EGF decreased the time to healing to 7-17 days (mean:10.9 days) compared with 9-21 days (mean: 12.3 days) for control donor sites. Madden et al showed that exudates from wounds occluded with a hydrocolloid dressing promoted keratinocyte proliferation.

DONOR SITE HEALING AND BACTERIA

Where healthy tissue exists and bacterial populations are non-invasive, wound healing proceeds in a normal fashion. In these cases, bacterial populations may stimulate the inflammatory response that initiates wound healing. Histologically observed invasion of viable tissue by pathogenic organisms distinguishes invasive wound sepsis from colonization.

Non-invasive bacterial populations may remain over the surface of the wound without impairing healing below[13]. The critical factor in wound healing appears to be the bacterial population in the wound, as opposed to the population over the surface of the wound. Bacterial populations vary over different parts of the body. This fact, plus concern for final cosmetic result, may influence donor site selection[3]. Preparation of the donor site area before harvest, as well as careful attention to hemostasis and clot removal from the bed after harvest, may be important for the control of microbial populations[11]. Depth of the donor area not only affects scar formation, but may also have a role in the incidence of infection [3]. As the depth of the wound increases,

healing is slowed, and the wound becomes more susceptible to bacterial contamination as the time to healing is prolonged. When colonization of the wound occurs, there may be enhancement of the initial inflammatory response caused by skin harvest. If this inflammatory response persists, the ensuing pathologic finding of edema and mediator-induced necrosis may predispose the underlying tissue to invasion.

Early after harvest, the inflammatory response in the surrounding tissue may mask the inflammatory response associated with bacterial colonization[12]. Hunt showed the cascade of inflammatory events associated with normal wound healing; however, the inflammatory response compounded by microorganisms may be severe and lead to destruction of adjacent tissue. Necrosis of tissue assists microbial invasion and conversion of the skin graft donor site to a full skin-thickness injury with a reported incidence of infection as high as 25%[2, 10, 32].

DONOR SITE DRESSINGS AND INFECTION

A donor site dressing should maximize the ability of the wound to heal without increasing the risk of local or systemic infection.

DONOR SITE DRESSINGS ARE DIVIDED INTO SEVERAL CATEGORIES:

OPEN, SEMI-OPEN, SEMI-OCCLUSIVE, AND OCCLUSIVE

As early as 1962, Winter CD showed that moist wounds healed faster than wounds left to dry out[36]. This observation has led the care of skin graft donor sites away from the conventional dry gauze dressings toward the semi-occlusive or occlusive dressings. Although these occlusive dressings provide moist environments for wound healing, there has been concern that occlusion of wounds would lead to increased infection17.However, Hutchinson and McGuckin. 2, in a review of 29 donor site

studies, showed an infection rate of only 2.7% in 594 occluded wounds versus an infection rate of 6.4% in 360 conventionally dressed wounds.

OCCLUSIVE TECHNIQUE

The early occlusive dressings consisted of a fine mesh gauze covered with an impermeable dressing; these were abandoned in favour of fine mesh gauze alone because of the potential for bacterial proliferation and difficulty in application to many areas, especially those other than extremities[32].

SEMI-OCCLUSIVE TECHNIQUE

The group of clear films often referred to as SAM dressings (synthetic adhesive moisture-vapour-permeable) was introduced for use on skin graft donor sites. They are also bacteria and liquid impermeable and so are considered semi-occlusive3. While the results of numerous studies have shown these dressings to promote more rapid and less painful healing, they tend to be severe-intensive, especially in large donor sites, because of the potential for large fluid collections. This problem often requires placement of a drain beneath the dressing at the time of initial application or, alternatively, frequent aspiration or changing of the dressing[32, 37].

OPEN TECHNIQUE

The open technique of leaving the wound uncovered is the least expensive of any dressing, but is quite painful and is associated with prolonged healing times[38].

SEMI-OPEN TECHNIQUE

Prior studies of fine mesh gauzes impregnated with various substances have described their ease of use and low cost, especially for large donor sites[38]. These dressings are

semi-Open. There is egress of fluid and bacteria through the fine mesh; as the dressing dries, fibrin from the wound bed causes temporary bonding of the dressing to the wound[2, 10]

Split thickness skin graft donor sites have been treated with open or closed dressings13. The open technique of donor dressing has been long abandoned in favor of the closed method since occlusive dressings have shown better results with shorter healing time, superior quality of the regenerated epithelium and more patient comfort. It has also shown the added advantage of protecting the donor site from desiccation, mechanical trauma and contamination. A more traditional method is dressing the donor site with a fine mesh gauze beneath a closed absorbent dressing. The gauze may be dry but is usually impregnated with bismuth, scarlet red or petroleum jelly. Though the gauze initially provides a moist environment it gradually becomes desiccated and an eschar forms which acts as a mechanical barrier and impairs cellular migration.

However, these dressings can also become permeable to bacteria if wound exudate soaks through the entire thickness of the dressing. Furthermore, movement of the donor site dressing produces shearing forces that may cause pain, dislodge the dressing and impair the migration of epithelial cells.

At the time of removal, the dressing is adherent and liable to damage the fragile regrown epithelium1,8. Studies have shown that a moist environment promotes healing in a partial thickness skin loss. The use of polyurethane film, a semi permeable dressing maintains a moist environment allowing diffusion of oxygen and water vapour while providing a barrier to the passage of wound exudates. It has claimed to reduce the healing time and donor site pain. However, it has proved

difficult to use as wound exudate collects beneath the film and is liable to leak out[1,40]. Other experiments have used Silicone gel sheets, also a semi permeable dressing with similar results.

BIOBRANE

Biobrane is a biocomposite of ultrathin semipermeable silicone membrane bonded to a flexible knitted nylon fabric. The two layers are covalently bonded to porcine collagen peptides, which increase wound adherence. The flexibility and stretch of Biobrane enable its application to many different donor site areas; its high-water vapor permeability minimizes fluid collections, and the ability to see through it permits ongoing evaluation of the wound.

A limited number of studies comparing Biobrane with more conventional donor site dressings have been showing with mixed results.

DUODERM

Duoderm is an oxygen-impermeable, hydrocolloid dressing, is being used extensively for treatment of dermal ulcers, burns and minor abrasions, and as a dressing for skin graft donor sites. It is composed of an outer layer of polyurethane foam that is impermeable to oxygen and water and an inner layer of hydrocolloid polymer complex that is occlusive and hydrophilic. Its oxygen impermeability has been shown to promote the rate of epithelialization and collagen synthesis and to decrease the pH of wound exudate, thus potentially reducing bacterial counts[3,11]. Because the dressing does not adhere to open wounds, it neither damages newly formed epithelium nor causes irritation or pain during dressing changes. The results of studies comparing

Duoderm with conventional fine mesh gauze have confirmed its potential clinical usefulness for skin graft donor sites with certain reservations [34,40,41]

OMNIDERM

Omniderm is a polyurethane Film, which is transparent, hydrophilic and highly permeable to water.

XEROFORM

A popular fine mesh gauze, inexpensive, easy to use and associated with a low infection rate. Results also confirm that re-epithelialization of donor sites covered with xeroform occurs in about ten days. However, Xeroform was more painful as a dressing than Biobrane or Duoderm. Patients complain most when the rolled gauze bandage was removed on the first postoperative day. Coagulum caused the Xeroform to stick to the gauze and removal was quite painful.

OPSITE

It is a polyurethane dressing. These dressings will provide a seal, thereby eliminating the risk of external infection as well as diminishing pain. In addition, these dressings have no pro-healing properties.

TEGADERM

Absorbent Clear Acrylic Dressing is a moisture-retentive, absorbent dressing which combines the benefits of highly absorbent dressings such as hydrocolloids, foams, alginates and hydrofibers with the transparency of thin film dressings.

HYDROCOLLOIDS

These promote healing, leaving donor sites soft, pink, supple and suitable for reharvesting, if necessary, within eight days (Doherty et al, 1986). They are simple to change and cause minimal disruption to new epithelium. The patient experiences increased comfort and healing rates and decreased pain. However, hydrocolloids can be costly and time consuming and require many dressing changes due to leakage, which can be offensive smelling and distressing for the patient.

CALCIUM ALGINATES

Attwood (1989) suggested these are inexpensive dressings, which increase haemostasis, comfort, speed of healing and quality of the new skin. They have been used quite widely for donor sites. They do have problems with drying out and adhering to the wound surface.

SOFT SILICONE WOUND CONTACT DRESSING (MEPITEL)

This has not been used widely for donor sites, mainly due to cost, which is significantly more than that for alginates or hydrocolloids. However, Mepitel is easier to remove and does not shed fibers into the wound. It has also been found to stop donor-site slippage (Wilkinson,1997).

FOAM DRESSINGS

There is a lack of research in the use of foam dressings to manage donor sites but their absorbency and comfort suggests they might have a place in this area. Wilkinson (1997) supports this and suggests that foams have a low adherence at the wound interface, can retain significant amounts of exudate and can be cut to size.

HYDROFIBRE DRESSINGS

Successful use of these dressings (Aquacel) and those impregnated with silver on donor sites have been reported (Barnea et al, 2004; Perlov et al 2001).

TISSUE ENGINEERED WOUND MATRIX COVERAGE

The advantage of the use of Wound Matrix dressing is that the dermis lost with the STSG is replaced with Wound Matrix as it incorporates. The Tissue Engineered Wound Matrix also contains all the active proteins and matrix components of dermis which can increase the rate of re-epithelialization. In addition, the use of a wound matrix results in immediate wound closure thereby protecting the remaining dermis.

Recent experiments have shown that biological dressings create the most physiological interface between the wound surface and the environment and permit the body's reparative and immune system to function most efficiently. Experiments have been carried out using porcine xenografts, amniotic membranes and collagen sheets.

However, both have shown poor results. Porcine xenografts showed a large percentage of abnormal healing due to sub epithelial incorporation and rejection and the amniotic membrane dressings showed a delayed healing. Collagen sheets have been used as a donor site dressing which comes close to being called an ideal donor site dressing. During the last decade, various new dressing materials developed, like calcium alginate, hydro-colloid membranes and fine mesh gauze. These have a disadvantage in that they become permeable to bacteria. Biological dressings like collagen on the other hand, create the most physiological interface between the wound surface and environment, and are impermeable to bacteria.

A prospective randomised clinical pilot study to **'compare the effectiveness of the biobrane synthetic wound dressing, with or without autologous cell suspension, to the local standard treatment regimen in paediatric scald injuries; 2011'**[42]. 15% of scald presentations in 12-month period was the eligibility criteria. 13 patients were recruited into the pilot study. early intervention was associated with a decreased time to healing with fewer dressing change less pain and better scar outcomes.

Randomized clinical trial of '**Autologous skin cell suspension for accelerating reepithelialisation of split thickness donor sites Z Hu D Guo et al 2017 BJS'**[4]**.**

106 patients were included 53 in each group. Median time to complete re-epithelization was 9.0 days in experimental group .it took 13 day in control group overall post-operative pain and itching scores were similar in both groups. Both patients and observers were satisfied with autologous skin cell suspension had been used.

Randomised clinical trial of '**autologous skin cell suspension combined with skin grafting for chronic wounds Z.C Hu D Chen et al Jan 2015'**[5]**.**

Out of 88 patients included in study ,44 in each group more patients achieved wound closure in the skin cell group than in the control group. Complete wound closure was observed at a median of 14% (12 to 16) days in the skin cell group and 20 days in control group, the skin cell group had significantly fewer complications. The autografted sites displayed better physical attributes and a reduced tendency for wound recurrence in the skin cell group.

MATERIALS AND METHODS:

SOURCE OF DATA:

All patients presenting to _____ _____ur and admitted patients in whom posted for skin grafting between October 2017 to June 2019.

METHOD OF COLLECTION OF DATA

Two groups were made, Group 1 receiving local injection of skin cell suspension and Group 2 conventional colloid dressing.

INCLUSION CRITERIA

- ✓ Men and women (non-pregnant) between 18 and 80 years
- ✓ Single donor site more than 20 cm^2
- ✓ Multiple donor site allocated treatment on all wounds but only largest donor site meeting inclusion criteria

EXCLUSION CRITERIA

- ✓ Malignancy
- ✓ Autoimmune disease
- ✓ Chemotherapy
- ✓ Corticosteroid
- ✓ Skin disease and local irradiation.

PROCEDURE

A Biopsy area of 1cm^3 is required to treat donor site size of 40cm^2. Skin biopsy is taken and Normal Saline (NS) is added into the tissue Homogenizer. The process is performed at a room temperature of 22-24 ^{0}C. Homogenizer is made to rotate at rate of 1200rpm. Autologous skin cell suspension is transferred to a 10 ml syringe and locally infiltrated in wound margin with 18 g needle at distance of 0.2 ml/cm. Local dressing is opened on 3rd day followed by alternate day dressing with NS.

Patient will be followed up to 12 weeks and photographs will be taken to evaluate physical appearance of donor site and quality of healing. Treatment outcome will be determined by median time of wound healing.

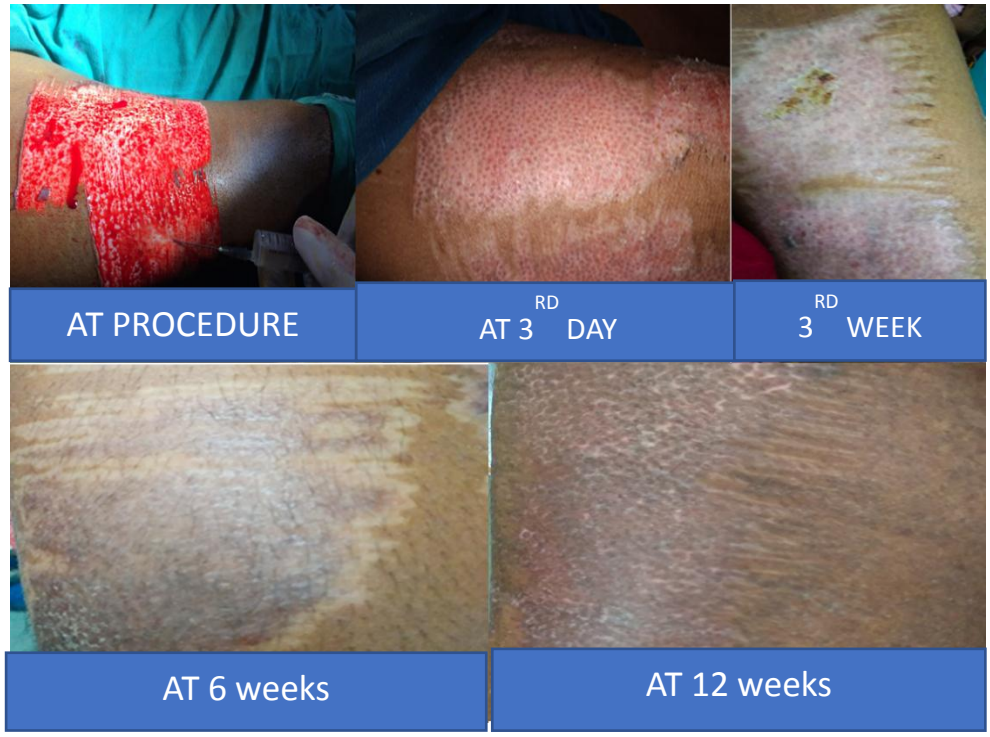


Figure 4 – Cases Group

Figure 5- Control Group

STATISTICAL ANALYSIS

All characteristics were summarized descriptively. For continuous variables, the summary statistics of mean± standard deviation (SD) were used. For categorical data, the number and percentage were used in the data summaries and diagrammatic presentation. Chi-square (χ^2) test was used for association between two categorical variables. The formula for the chi-square statistic used in the chi square test is:

$$\chi_c^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

The subscript "c" are the degrees of freedom. "O" is observed value and E is expected value.

The difference of the means of analysis variables between two independent groups was tested by unpaired t test. The t statistic to test whether the means are different can be calculated as follows:

$$t = \frac{(\overline{x_1} - \overline{x_2}) - (\mu_1 - \mu_2)}{\sqrt{\frac{{s_1}^2}{n_1} + \frac{{s_2}^2}{n_2}}}$$

where $\bar{x}_1$ = mean of sample 1

$\bar{x}_2$ = mean of sample 2

n_1 = number of subjects in sample 1

n_2 = number of subjects in sample 2

${s_1}^2$ = variance of sample 1 = $\frac{\Sigma(x_1 - \bar{x}_1)^2}{n_1}$

${s_2}^2$ = variance of sample 2 = $\frac{\Sigma(x_2 - \bar{x}_2)^2}{n_2}$

If the p-value was < 0.05, then the results were considered to be statistically significant otherwise it was considered as not statistically significant. Data were analyzed using SPSS software v.23.0. and Microsoft office 2007.

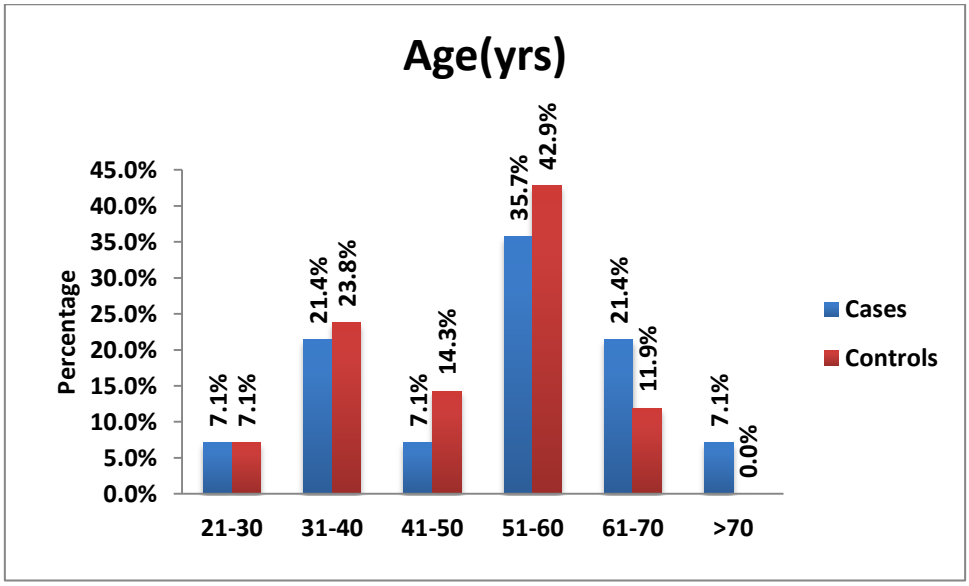
RESULTS

A total of 84 patients were included the study and were randomly divided into two groups ,42 patients in skin cell suspension injection into donor site group and 42 patients in colloid dressing group.

Table1: DISTRIBUTION OF AGE BETWEEN CASES AND CONTROLS

Age (yrs.)	Cases		Controls		P- value
	N	%	N	%	
21-30	3	7.1%	3	7.1%	
31-40	9	21.4%	10	23.8%	
41-50	3	7.1%	6	14.3%	
51-60	15	35.7%	18	42.9%	0.361
61-70	9	21.4%	5	11.9%	
>70	3	7.1%	0	0.0%	
Total	42	100.0%	42	100.0%	

Figure6: DISTRIBUTION OF AGE BETWEEN CASES AND CONTROLS

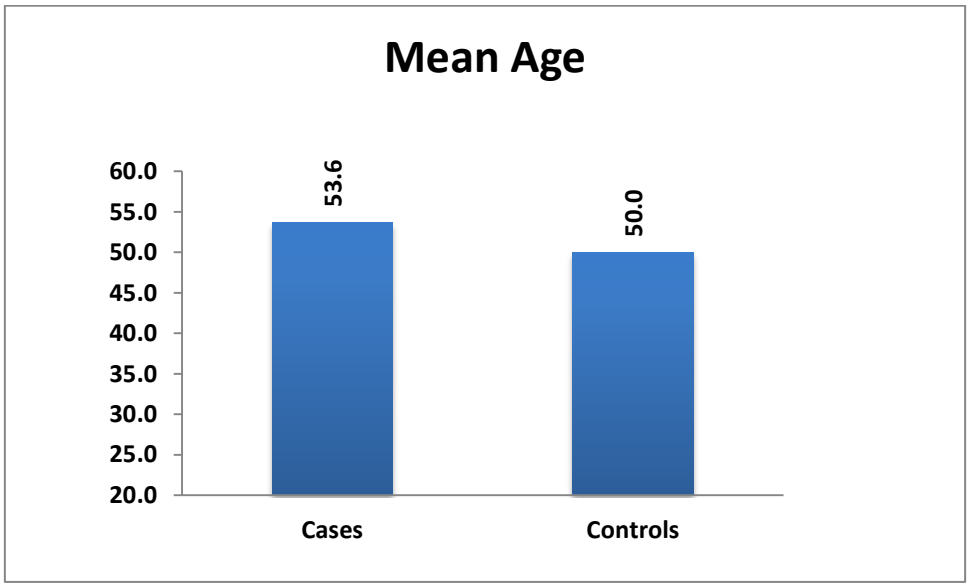


The mean age in cases group were 35.7% and in control group were 42.9%. The most of the patients were in between51-60 years. In case group 15 patients and 18 patients control group were between 50-60 years.

Table 2: MEAN AGE BETWEEN CASES AND CONTROLS

Parameters	Cases		Controls		p value
	Mean	SD	Mean	SD	
Age(yrs)	53.6	14.3	50.0	12.4	0.209

Figure7: MEAN AGE BETWEEN CASES AND CONTROLS

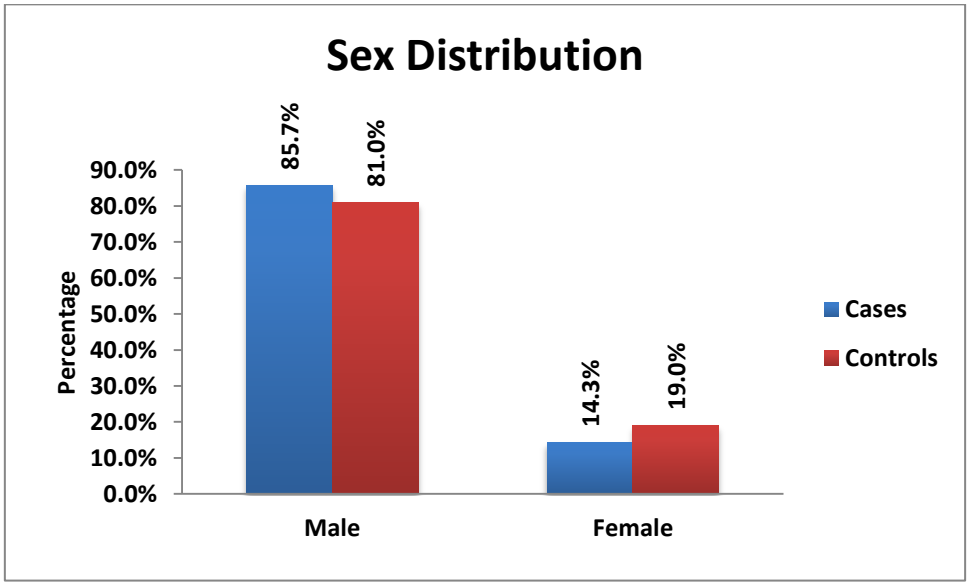


The mean age group of cases were 53.6% and control group was 50 years. P-value was found to be 0.209 which was statistically insignificant. Most of the patient were in the study were above 50 year.

Table3: DISTRIBUTION OF SEX BETWEEN CASES AND CONTROLS

Sex	Cases		Controls		P-value
	N	%	N	%	
Male	36	85.7%	34	81.0%	
Female	6	14.3%	8	19.0%	0.558
Total	42	100.0%	42	100.0%	

Figure8: DISTRIBUTION OF SEX BETWEEN CASES AND CONTROLS

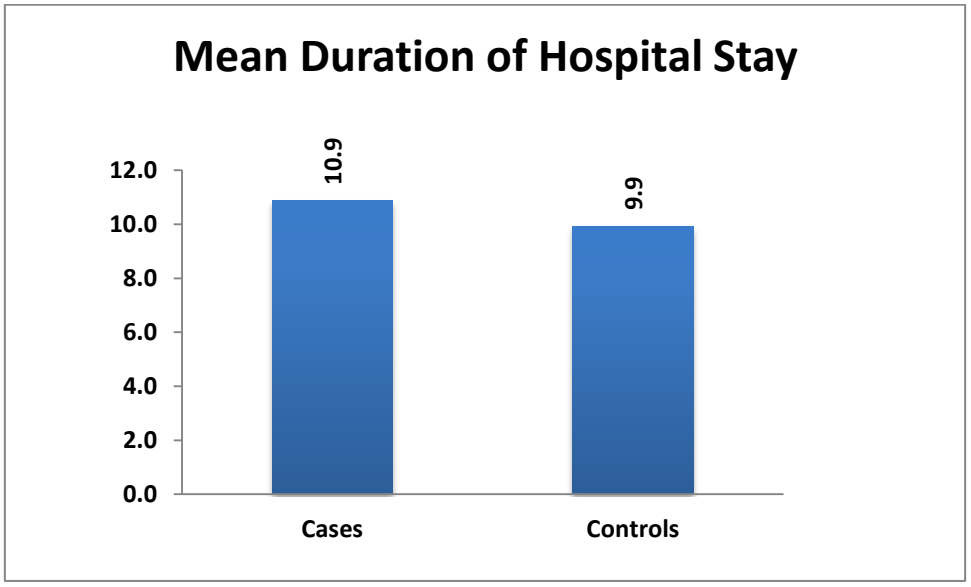


In cases the number of males were 36(85.7%) male patients and female patients were 6(14.3%). In control group male patients were 34(81%) and females were 8(19%) females. When compared to ratio male to females, males were higher than females 70:14 in study group.

Table 4: MEAN DURATION OF HOSPITAL STAY BETWEEN CASES AND CONTROLS

Parameters	Cases		Controls		p value
	Mean	SD	Mean	SD	
Duration of Hospital Stay	10.9	7.1	9.9	3.2	0.431

Figure9: MEAN DURATION OF HOSPITAL STAY BETWEEN CASES AND CONTROLS

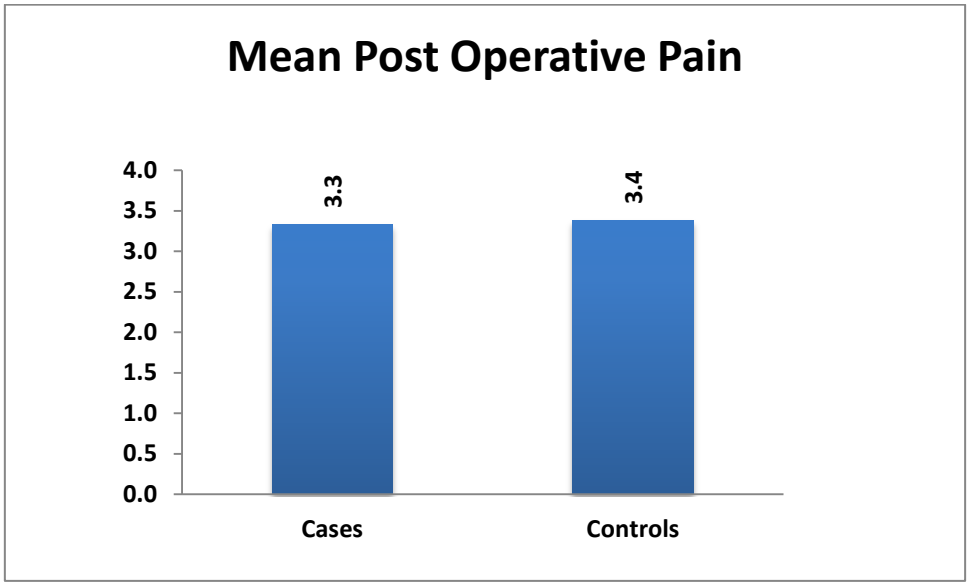


In cases group the hospital stay mean duration was of 10.9days and in control group was 9.9 which 0.431 statistically insignificant.

Table5: POST OPERATIVE PAIN BETWEEN CASES AND CONTROLS

Parameters	Cases		Controls		P-value
	Mean	SD	Mean	SD	
Post OP Pain	3.3	0.5	3.4	0.5	0.654

Figure10: POST OPERATIVE PAIN BETWEEN CASES AND CONTROLS



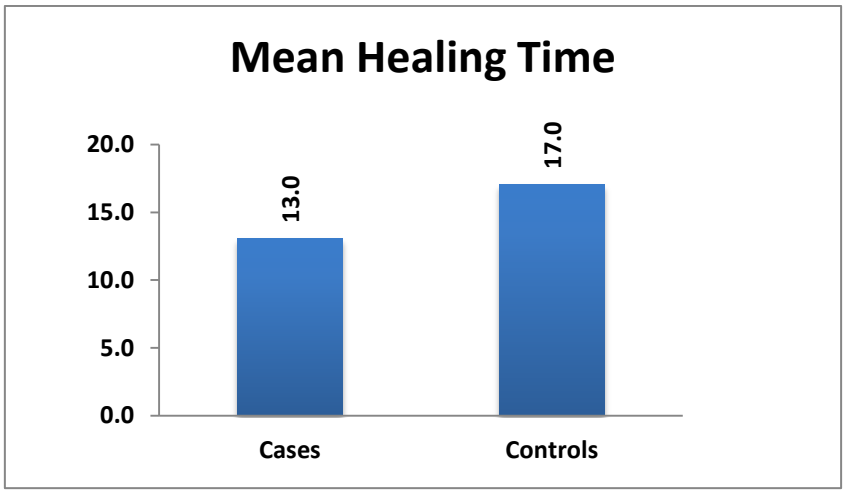
In cases group based on the visual pain analogue scale, pain in the cases group was 3.3days and in control group was 3.4 days which was statistically insignificant P value 0.654.

Table6: HEALING TIME BETWEEN CASES AND CONTROLS

Parameters	**Cases**		**Controls**		**P-value**
	Mean	**SD**	**Mean**	**SD**	
Healing Time	13.0	1.3	17.0	1.0	**<0.001***

Note: * significant at 5% level of significance (p<0.05)

Figure11: HEALING TIME BETWEEN CASES AND CONTROLS

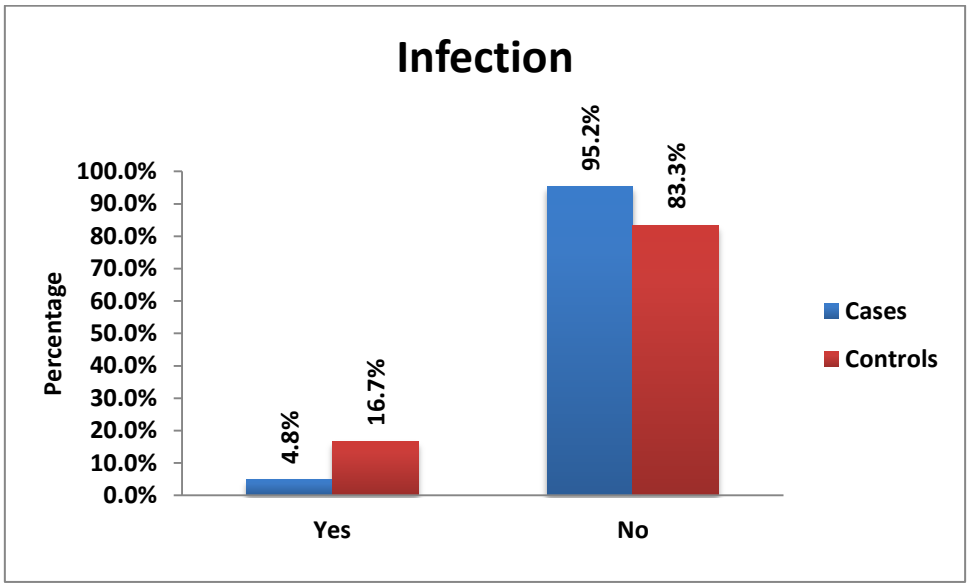


The mean healing time in the cases was 13 days, whereas in control group, it was found to be 17 days, P-value was very significant P value **0.001** which states that healing time was faster in cases compared to control group.

Table7: DISTRIBUTION OF INFECTION BETWEEN CASES AND CONTROLS

Infection	Cases		Controls		P-value
	N	%	N	%	
Yes	2	4.8%	7	16.7%	0.078
No	40	95.2%	35	83.3%	
Total	42	100.0%	42	100.0%	

Figure12: DISTRIBUTION OF INFECTION BETWEEN CASES AND CONTROLS



The infection rate in cases were 2(4.8%) where as in control group 7 (16.7%) patients.

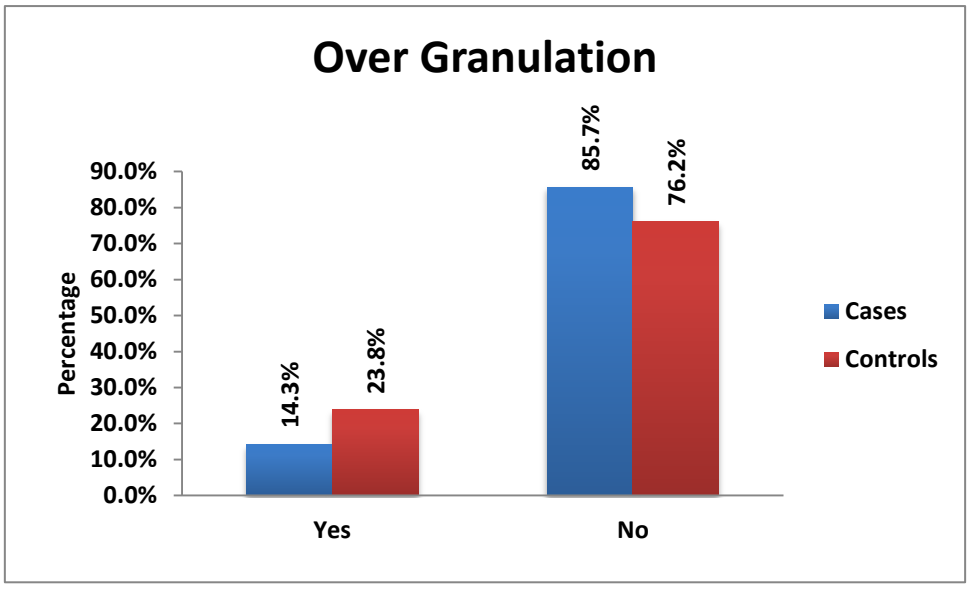
The non-infected in case were 40 (95.2%) and in control group 35 patients (83.3%).

The total 75 patients were non infected in study group.

Table8: DISTRIBUTION OF OVER GRANULATION BETWEEN CASES AND CONTROLS

Over Granulation	Cases		Controls		p value
	N	%	N	%	
Yes	6	14.3%	10	23.8%	0.266
No	36	85.7%	32	76.2%	
Total	42	100.0%	42	100.0%	

Figure13: DISTRIBUTION OF OVER GRANULATION BETWEEN CASES AND CONTROLS

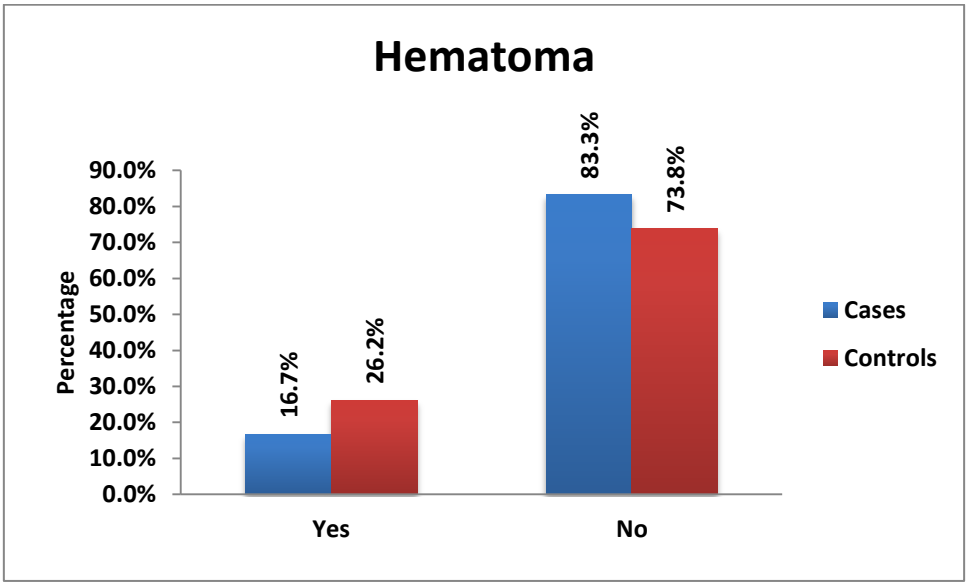


The over granulation in cases were 6 patients (14.3%) normal patients were36 (85.7%). In control group over granulation patients were 10 (8%). Whereas normal patients were 32(76.2%). The total over granulation patients were 16 and normal patients were 68 in whole study, which was statistically insignificant.

Table9: DISTRIBUTION OF HEMATOMA BETWEEN CASES AND CONTROLS

Hematoma	Cases		Controls		p value
	N	%	N	%	
Yes	7	16.7%	11	26.2%	0.287
No	35	83.3%	31	73.8%	
Total	42	100.0%	42	100.0%	

Figure14: DISTRIBUTION OF HEMATOMA BETWEEN CASES AND CONTROLS

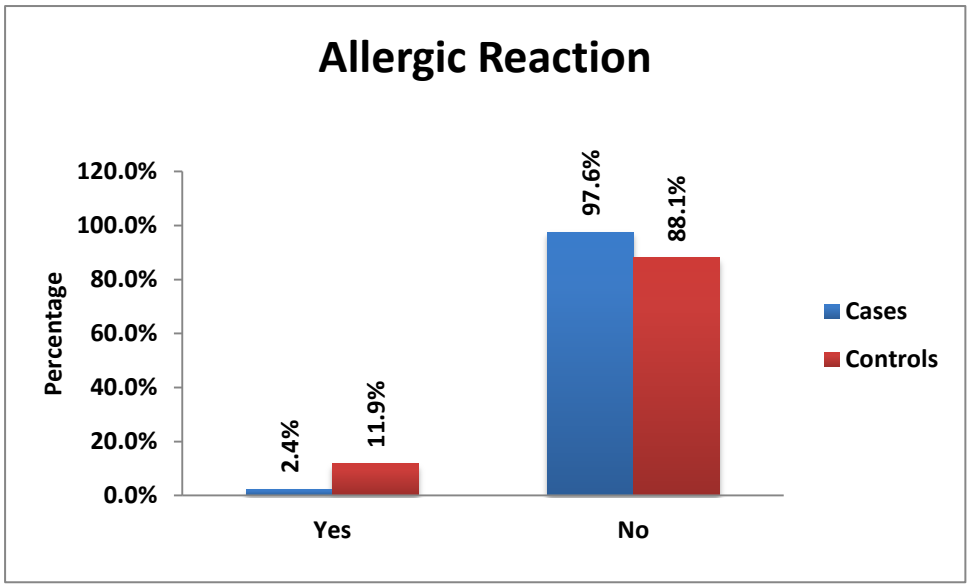


The hematoma in cases and control group 7 (16.7%) and 11((26.2%) patients respectively. In cases group normal patients were 35 (83.3%), in control group 31 (73.8%), which was statistically insignificant.

Table10: DISTRIBUTION OF ALLERGIC REACTION BETWEEN CASES AND CONTROLS

Allergic Reaction	Cases		Controls		P- value
	N	%	N	%	
Yes	1	2.4%	5	11.9%	0.090
No	41	97.6%	37	88.1%	
Total	42	100.0%	42	100.0%	

Figure15: DISTRIBUTION OF ALLERGIC REACTION BETWEEN CASES AND CONTROLS

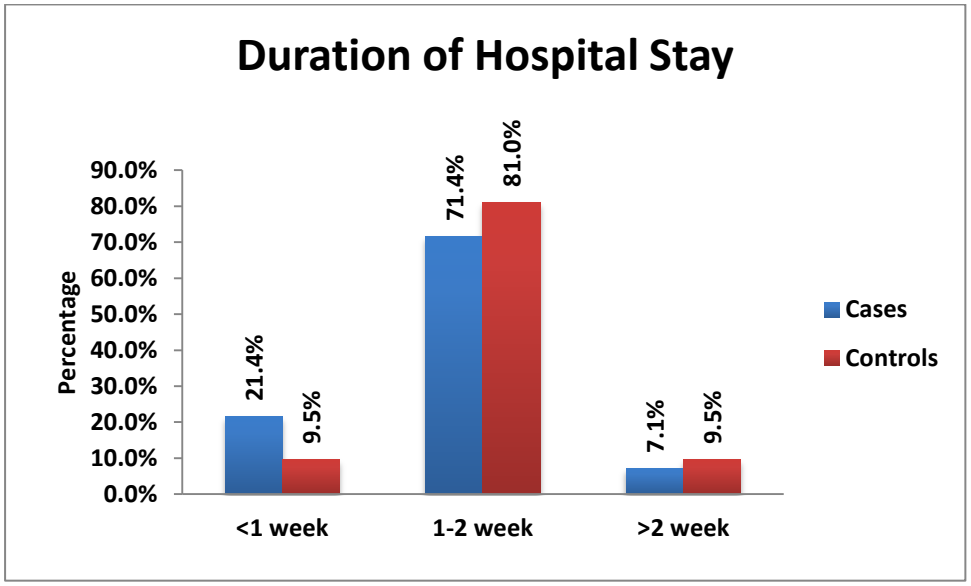


Most of the patients in cases group were non allergic 41 (97.6%), only 1(2.4%) patient was allergic. Where as in control group 37 (88.1%) patients were normal and 5 (11.9%) patients were allergic which was statistically insignificant.

Table11: DURATION OF HOSPITAL STAY BETWEEN CASES AND CONTROLS

Duration of Hospital Stay	Cases		Controls		P-value
	N	%	N	%	
<1 week	9	21.4%	4	9.5%	0.314
1-2 week	30	71.4%	34	81.0%	
>2 week	3	7.1%	4	9.5%	
Total	42	100.0%	42	100.0%	

Figure16: DURATION OF HOSPITAL STAY BETWEEN CASES AND CONTROLS



In cases group 9 (21.4%) stayed less than a week. Maximum patients were stayed between 1-2weeks, 30 patients (71.4%).3 patients (7.1%) stayed more than 2weeks in the hospital. In control group 4 patient (9.5%) stayed less than a week whereas 34 patients between 1-2 weeks (81%) and 4 patients (9.5%) stayed more than 2 weeks. Compared to cases and control group statistically insignificant.

DISCUSSION

In our study, it was observed that in case group 15 patients (35.97%) and in control group 18 patients (42.9 %) were between 51-60 years

In Z. HU et al [4]most of the patients were between 61-80 years in cases, 24 patients (45 %) and in control group 20 patients (38%)

In Z.C. Hu et al[5]the mean age in cases group was 48 years and in control group 50 years.

F. wood et al[42] cases group the mean age of patients was 49 years and in control group 50 years.

Our study was in line with the above-mentioned studies

In our study, out of 84 patients, 70 (83.3 %) were male, and 14 (16.6 %) were females. Among the male patients, 36 belonged to case group and among 14 female patients, 6 were in the case group.

In Z. HU et al[4] out of 106 sample size, 76 (71.6 %) were male and 30 (28.3 %) were females.

Out of 76 male patients 40 were from case group and 36 from the control and among the total female patients, 13 were from the case group and 17 from control.

In Z.C. Hu et al[5] out of 88 sample size, 58 (65.9 %) were male and 30 (34 %) were females.

Out of 88 male patients 30 were from case group and 28 from the control and among the total female patients, 14 were from the case group and 16 from control.

Our findings were in concordance with the above studies.

There was no statistical difference between the study and control group.

In our study, the mean post-operative pain in case group was 3.3 and in control group 3.4 % as per visual analogue pain scale.

In Z. HU et al [4] the mean post-operative pain was 1.7 and in control group 1.6

In F. wood et al [42] the mean post-operative pain was 3 and in control group 5.5

In our study, it was found that the mean healing time in case group was 13 days and in control group 17 days.

In Z. HU et al[4] concluded that the mean healing time in case group was 9 days and in control group 13 days.

In Z.C. Hu et al[5] concluded that the mean healing time in case group was 14 days and in control group 20 days.

However, in a study conducted by F. Wood et al[42], it was seen that the mean healing time in study group was 15 days and in control group 34 days.

Our findings are in line with the above compared studies.

Out of the 84 patients included in our study, 9 patients (10.7 %) were infected, among them, two cases were from study group and 7 from control group.

In Z. HU et al[4], only two patients from the control group were infected.

In Z.C. Hu et al[5], one patient from the case group and three from the control group were infected.

F. wood et al[42] one case from each group were infected.

CONCLUSION

Autologous skin cell suspension in donor site of spilt skin thickness graft with colloid dressing. is easier and faster. It has been observed that, healing with autologous skin cell suspension and hydrocolloid dressing was faster when compared with the control group (only hydrocolloid dressing) and the procedure is easy to perform with minimal complications.

It also does not require special training to perform. The procedure is also cost effective and safe in terms of decreased hospital stay and faster healing.

SUMMARY:

In autologous skin cell suspension in donor site of spilt skin thickness graft with colloid dressing main aim is faster healing of donor site with any adverse events.

Our goal is to assess the safety and efficacy of skin cell suspension injecting into the donor site compared to only hydrocolloid dressing.

Period of study is from October 2017 to June 2019, with sample size 84. Autologous skin cell suspension injecting group was assessed by Local dressing is opened on 3rd day followed by alternate day dressing with NS. Patient will be followed up to 12 weeks and photographs will be taken to evaluate physical appearance of donor site and quality of healing.

Treatment outcome will be determined by median time of wound healing. There was no difference between the groups compared with respect to Age and Sex. Thus, we can conclude that the current research hypothesis of skin cell suspension injected into the donor site healed by 13 days where as control group 17 days which was statistically significant P value of <0.001.so we can conclude that faster healing in case group with minimal adverse events .

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ANNEXURE I – ETHICAL CLEARANCE CERTIFICATE

12. ANNEXURE II – SAMPLE INFORMED CONSENT FORM:

TITLE OF THE PROJECT : **AUTOLOGOUS SKIN CELL SUSPENSION FOR ACCELERATING RE-EPITHELIZATION OF SPLIT THICKNESS DONOR SITES.**

PG GUIDE **M.S. General Surgery**

Associate professor

Department of general surgery

PRINCIPAL INVESTIGATOR :

POST GRADUATE

DEPARTMENT OF GENERAL SURGERY

PURPOSE OF RESEARCH:

I have been informed that this study is conducted to compare and evaluate the efficacy of Autologous skin cell suspension injecting into the donor site with hydrocolloid dressing and only hydrocolloid.

PROCEDURE:

I am aware that in addition to routine care received I will be asked series of questions by the investigator. I have been asked to undergo the necessary investigations and treatment, which will help the investigator in this study.

Patients who met the inclusion criteria were randomly assigned a Study group (Group A) or Control group (Group B).

RISK AND DISCOMFORTS:

I understand that I may experience some pain and discomforts during the examination or during my treatment. This is mainly the result of my condition and the procedures of this study are not expected to exaggerate these feelings which are associated with the usual course of treatment.

BENEFITS:

I understand that my participation in the study will help to predict to evaluate the faster healing of donor site with Autologous skin cell suspension compared to hydrocolloid dressing at donor sites.

CONFIDENTIALITY:

I understand that the medical information produced by this study will become a part of hospital records and will be subject to the confidentiality. Information of sensitive personal nature will not be part of the medical record, but will be stored in the investigations research file.

If the data are used for publication in the medical literature or for teaching purpose, no name will be used and other identifiers such as photographs will be used only with special written permission. I understand that I may see the photograph before giving the permission.

REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study to **Dr.** in the Department of General Surgery who will be available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of the study, which might influence my continued participation. A copy of this consent form will be given to me to keep for careful reading.

REFUSAL FOR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and that I may refuse to participate or may withdraw consent and discontinue participation in the study at any time without prejudice. I also understand that may terminate my participation in the study after he has explained the reasons for doing so.

INJURY STATEMENT:

I understand that in the unlikely event of injury to me resulting directly from my participation in this study, if such injury were reported promptly, the appropriate treatment would be available to me. But no further compensation would be provided by the hospital. I understand that by my agreements to participate in this study and not waiving any of my legal rights.

I have explained to _____the purpose of the research, the procedures required and the possible risks to the best of my ability.

_____ _____

Date

(Investigator)

STUDY SUBJECT CONSENT STATEMENT:

I confirm has explained to me the purpose of research, the study procedure, that I will undergo and the possible discomforts as well as benefits that I may experience in my own language. I have been explained all the above in detail in my own language and I understand the same. Therefore, I agree to give consent to participate as a subject in this research project.

_____ _____

(Participant) Date

_____ _____

(Witness to signature) Date

ANNEXURE III – PROFORMA

PROFORMA

CASE NO:

- Name: IP No:
- Age/sex: DOA:
- Occupation:
- Address: DOD:

- CHIEF COMPLAINTS:

WOUND:

- Mode of Onset
- Duration
- Number
- Site
- Size and Extent
- Associated Pain
- Discharge
- Others
- Any Associated Disease
- Past History of Similar Wound
- Personal History

PAST HISTORY:

- Diabetes Mellitus
- Hypertension
- Peripheral vascular disease
- HIV

PERSONAL HISTORY:

- Diet
- Sleep
- Appetite
- Bowel & bladder

7. GENERAL PHYSICAL EXAMINATION:

- Mental Status — Weight:
- Built — Height:
- Nourishment — BMI:
- Hydration status
- Pallor
- Icterus
- Cyanosis
- Clubbing
- Edema
- Cervical Lymph Nodes
- Pulse
- Blood Pressure
- Respiration
- Temperature
- Any Obvious Deformity

A. EXAMINATION OF LYMPH NODES

B. EXAMINATION OF VASCULAR SYSTEM

C. EXAMINATION OF NERVE SUPPLY OF THE LIMB

SYSTEMIC EXAMINATION

PER ABDOMEN:

Inspection:

Palpation:

Percussion:

Auscultation:

R*ERSPRATORY SYSTEM*:

Inspection

Palpation

Percussion

Auscultation

CARDIO-VASCULAR SYSTEM:

Inspection

Palpation

Percussion

Auscultation

C*ENTRAL NERVOUS SYSTEM:*

Higher Mental functions

Diagnosis

INVESTIGATIONS:

- Hemoglobin
- Total Count
- Differential Count
- ESR
- Serum Albumin
- Blood Urea,
- Serum Creatinine
- BLOOD SUGAR (RBS, FBS, PPBS)
- Urine For Ketone Bodies (If Diabetic)

- HbA_1C (If Diabetic)
- Urine Routine
- ECG
- Chest X-ray
- Culture Sensitivity of Discharge
- X-Ray of bone Or Joint Involved
    - Post Procedure, Once in 3 weeks for next 3 months.

COMMENTS:

PRE-TREATEMENT MEASUREMENTS:

SERIAL MEASUREMENTS DURING THE TREATEMENT:

FINAL MEASUREMENTS:

PROGRESSION OF HEALING: (In terms Of median time)

INFERENCE:

MASTER CHART

name	AGE	sex	IP NO	DOS	DOD	POST op pain	infection	over granulation	hemtoma	allergic reaction	median time
mutappa	85	male	7108/18	16-Mar	24-Mar	3	No	NO	No	NO	10
nagappa	65	male	10522/18	14-Apr	20-Apr	3	Yes	No	Yes	NO	11
shivappa	60	male	12963/18	28-Apr	05-May	3	No	Yes	No	NO	11
nagappa	80	male	24928/18	08-Aug	18-Aug	3	No	Yes	No	NO	13
shivanand	53	male	29738/18	06-Sep	20-Sep	3	Yes	No	NO	NO	12
danappa	38	male	25479/18	09-Oct	20-Oct	3	No	No	Yes	NO	11
ganapati	62	male	33175/18	13-Oct	23-Oct	3	No	No	NO	NO	13
bhimaraya	62	male	32961/18	10-Oct	22-Oct	3	No	No	No	NO	12
hanamanth	60	male	28688/18	11-Sep	19-Sep	3	No	No	No	NO	13
kalesab	38	male	25959/18	20-Aug	03-Sep	3	No	No	No	NO	12

laxman	70	male	25517/18	10-Aug	23-Aug	4	No	No	No	NO	13
iranna	40	male	24667/18	10-Aug	23-Aug	3	No	Yes	Yes	yes	12
siddappa	64	male	27246/18	13-Sep	20-Sep	4	No	No	No	NO	12
sunil	47	male	29889/18	10-Sep	20-Sep	3	No	No	No	NO	13
chandrappa	80	male	30253/18	06-Sep	18-Sep	4	No	No	No	NO	13
bandagisab	70	male	27277/18	06-Sep	13-Sep	4	No	No	No	NO	12
sangappa	48	male	31091/18	25-Sep	12-Oct	3	No	Yes	NO	NO	13
sangayya	55	male	31511/18	29-Sep	10-Oct	4	No	No	No	NO	12
basavaraj	59	male	33464/18	12-Oct	19-Oct	4	No	No	Yes	NO	13
mustan	40	male	27342/18	15-Oct	20-Oct	3	No	No	No	NO	12
prakash	65	male	6063/19	11-Mar	20-Mar	3	No	No	No	NO	13
uday	32	male	3377/19	22-Feb	03-Mar	4	No	No	No	NO	12
prakash	65	male	6069/19	11-Mar	22-Mar	3	No	No	No	NO	13
prabhu	32	male	6500/19	15-Mar	28-Mar	3	No	No	No	NO	13
suresh	28	male	5588/19	18-Mar	30-Mar	3	No	No	Yes	NO	12

rajashekhar	45	male	5398/19	27-Feb	03-Mar	4	No	No	No	NO	14
shoba	30	female	2931/19	27-Feb	05-Mar	3	No	No	No	NO	15
mahantesh	53	male	4302/19	09-Feb	15-Mar	3	No	No	No	NO	16
huligeppa	55	male	4096/19	08-Mar	20-Mar	3	No	No	Yes	NO	15
laxmi	56	female	5926/19	14-Mar	20-Mar	4	No	Yes	NO	NO	14
mahadevi	36	female	6840/19	18-Mar	24-Mar	3	No	No	No	NO	12
shamaraya	60	male	7896/19	22-Mar	31-Mar	4	No	No	Yes	NO	14
vishwa	26	male	7055/19	28-Mar	10-Apr	4	No	No	NO	NO	15
gopal	60	male	4525/19	07-Mar	18-Mar	3	No	Yes	NO	NO	14
shaila	30	female	10191/19	11-Apr	25-May	3	No	No	No	NO	15
laxmi	55	female	11865/19	25-Apr	05-May	4	No	No	No	NO	14
kallappa	55	male	11816/19	02-May	12-May	3	No	No	No	NO	13
gururaj	40	male	11388/19	29-Apr	05-May	3	No	No	No	NO	14
pandurang	60	male	18424/19	19-Jun	29-Jun	3	No	No	No	NO	15
shrishail	65	male	18437/19	19-Jun	28-Jun	3	No	No	No	NO	13

ameerbee	55	female	19384/19	27-Jun	02-Jul	4	No	No	No	NO	14
mahadevappa	40	male	19356/19	27-Jun	03-Jul	4	NO	NO	NO	NO	15

CONTROLS

manappa	65	male	7992/18	20-Mar	28-Mar	4	NO	NO	NO	NO	14
basavaraj	31	male	10781/18	10-Apr	20-Apr	3	NO	NO	NO	NO	16
anand singh	40	male	9463/18	11-Apr	23-Apr	4	NO	NO	NO	NO	17
mallikarjun	50	male	10656/18	13-Apr	20-Apr	4	NO	YES	NO	NO	18
siddappa	65	male	11617/18	20-Apr	05-May	4	NO	NO	YES	NO	15
mallanagoud	28	male	20012/18	24-Jun	10-Jul	3	NO	NO	NO	NO	18
shantayya	48	male	24061/18	08-Aug	20-Aug	3	NO	YES	YES	NO	19
dundappa	45	male	24859/18	14-Aug	22-Aug	3	YES	NO	NO	NO	17
maulasab	60	male	27514/18	30-Aug	09-Sep	4	NO	YES	NO	NO	18
ravi	40	male	31831/18	19-Sep	28-Sep	3	NO	NO	NO	NO	16
saidusab	55	male	33204/18	27-Sep	04-Oct	3	YES	NO	YES	NO	17
husenasab	60	male	33810/18	04-Oct	14-Oct	4	NO	NO	NO	NO	16
siddappa	58	male	41781/18	09/01/2019	18-Jan	3	YES	YES	NO	YES	16

mallappa	54	male	579/19	24-Jan	31-Jan	3	NO	NO	YES	NO	18
mantesh	33	male	404/19	20-Jan	03-Feb	4	NO	NO	NO	NO	16
shantabai	60	female	425/19	09-Jan	13-Jan	3	NO	NO	NO	NO	17
kallawwa	50	female	444/19	11-Feb	18-Feb	4	NO	NO	NO	YES	18
shivappa	57	male	1593/19	13-Feb	20-Feb	3	YES	NO	YES	NO	17
mahesh	27	male	3971/19	21-Feb	28-Feb	3	NO	YES	NO	NO	18
shivappa	60	male	1543/19	20-Feb	28-Feb	3	NO	NO	NO	NO	17
yallappa	50	male	4289/19	09-Feb	18-Feb	4	NO	NO	NO	YES	17
bhimappa	25	male	7587/19	14-Mar	20-Mar	3	NO	NO	YES	NO	18
basappa	40	male	4409/19	25-Feb	10-Mar	3	YES	YES	NO	NO	16
laxmi	65	female	4403/19	07-Mar	20-Mar	3	NO	NO	NO	NO	19
huligeppa	55	male	4096/19	08-Mar	20-Mar	4	NO	NO	NO	YES	18
lalamshaik	60	male	6066/19	18-Mar	30-Mar	3	NO	NO	YES	NO	17
suresh	35	male	5588/19	18-Mar	31-Mar	3	NO	NO	NO	NO	16
irayya	33	male	8817/19	31-Mar	08-Apr	3	NO	YES	NO	NO	17

bharati	64	female	7569/19	28-Mar	03-Apr	4	NO	NO	YES	YES	18
eshwarappa	60	male	12764/19	11-Apr	18-Apr	3	NO	NO	NO	NO	17
suresh	55	male	9499/19	31-Mar	10-Apr	3	NO	NO	NO	NO	18
chandrakant	60	male	6908/19	27-Mar	08-Apr	4	NO	NO	YES	NO	17
ramesh	35	male	9966/19	16-Apr	25-Apr	3	NO	NO	NO	NO	18
kalesh	34	male	5558/19	26-Apr	05-May	3	NO	YES	NO	NO	17
arjunsingh	40	male	12238/19	29-Apr	05-May	3	NO	NO	YES	NO	16
sulochana	54	male	13327/19	01-May	20-May	4	YES	NO	NO	NO	17
laxmibai	54	female	11365/19	01-May	16-May	4	NO	YES	NO	NO	16
shantabai	60	female	42504/19	01-Jun	11-Jun	3	NO	NO	NO	NO	17
shankaragouda	70	male	14692/19	20-May	30-May	3	NO	NO	NO	NO	18
durgawwa	60	female	14309/19	16-May	30-May	4	NO	NO	NO	NO	17
shivanand	43	male	16300/19	25-May	01-Jun	3	NO	YES	NO	NO	16
shusmita	26	female	19353/19	20-Jun	30-Jun	4	YES	NO	YES	NO	18