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# Clinical and Histological Evaluation of Acellular Allograft Dermal Matrix in Full-Thickness Burns

MOHAMAD R. EL-HADIDY, M.D. and AZZA R. EL-HADIDY, Ph.D.

*The Department of Plastic & Reconstructive Surgery and Histology & Cytology, Faculty of Medicine, Mansoura University.*

## ABSTRACT

Split-thickness, unmeshed skin grafts potentially inhibit wound contraction but not to the same degree as do full-thickness skin grafts. Thus, the presence of normal dermis was an important factor in the control of wound contraction and limitation of scar formation. The aim of this study was to evaluate the ability of an acellular dermal matrix processed from human allograft skin (AlloDerm) in the treatment of deep partial-thickness and full-thickness burn injury. Deep burn wounds in 11 patients (their ages range between 9 and 45 years and their mean total burned body surface area was 30%) were excised to subcutaneous fat, or fascia. Samples of AlloDerm were examined histologically to confirm its morphological properities. Following rehydration, AlloDerm was placed over the test sites, which were then covered with ultra thin split-thickness autograft. The control site was grafted with ultra thin split-thickness autograft alone. The clinical observations of "take" were confirmed with histological evaluation of the biopsies, which exhibited fibroblast cells infiltration, neovascularization and neoepithelization without evidence of rejection. Wound assessment over time showed that thin split-thickness autografts plus allograft dermal matrix were similar to thicker split-thickness autografts. In conclusion, AlloDerm acellular dermal graft conserve as dermal transplant for deep-partial and full thickness burn.

## INTRODUCTION

Early excision and grafting of burns in patients with extensive thermal injuries have shorted hospitalization times, decreased the number of infectious complications and have probably increased patient survival **[1-6]**. Full-thickness burn wounds are frequently closed with meshed and expanded split-thickness skin grafts (MESTSGs) because of the lack of available unburned skin to serve as sites for autografts. There is no dermis beneath the healed graft interstices as the MESTSG interstices close by means of epithelization over the full-thickness wound surface.The lack of dermis, extensive remodeling for extracellular matrix and differentiation of fibroblasts into myofibroblasts may contribute to hypertrophic scaring and wound contraction and to instability of the skin, for example, basement membrane structures are slow to form in the absence of dermal tissue. Split-thickness, unmeshed skin grafts partially inhibit wound contraction but not be the same degree as do full-thickness skin grafts. Thus, the presence of normal dermis has been postulated to be an important factor in the control of wound contraction and limitation of scar formation **[7-9]**.

Harvesting too much dermis contributes to donor site morbidity, including pain, infection, scarring and in some patients, keloid formation. This is particularly a problem in massive burn injuries, where limited donor sites must be recropped and in children and the elderly, who have thin skin.

The potential use of allograft donor skin as a permenant skin replacement in full-thickness burns is limited by its immunogenic properties. Allograft skin grafts will routinely take to a full-thickness wound, but are ultimately rejected **[10]**. This immune response to allograft skin is directed primarily against the cells of the epidermis **[11]** and the endothelial and fibroblast cells in the dermis **[11,12]**. The non-cellular components of dermis, consisting primarily of extracellular matrix proteins and collagen, have been shown to be relatively non-immunogenic **[13]**. The complexity of removing the immunogenic cells from the non-immunogenic dermis of allograft skin has previously restricted its use to temporary coverage of full-thickness burns **[14,15]**.

The concept of permanent transplantation of a non-immunogenic, extracellular tissue matrix has been clearly illustrated with the more robust matrix of bone. Freez dried bone allografts have been successfully used as permanent bone implants for many years [16]. The cells in these allografts are destroyed during the freez-drying process, but the structural organization of the extracellular matrix remains intact. These allografts provide an environment for osteogenic cell repopulation and osteoinduction with subsequent matrix remodelling and new bone formation [17].

The AlloDerm (Life Cell Corp, The Woodlands, Texas) process is designed to reduce the immunogenicity of allograft dermis by removing the cells that contain the antigenic targets for rejection, while maintaining the native structure and composition of the dermal matrix, including the basement membrane complex (BMC). Therefore, in this study we try to evaluate the histological character of the AlloDerm as non-immunogenic acellular dermal matrix and its clinical application to function as a permenant dermal transplant and potentially enhance the quality of skin grafts applied to full-thickness and deep partial thickness burns, while reducing donor site trauma and complications.

## MATERIAL AND METHODS

This study was carried out on 11 patients (7 males and 4 females). Whose ages ranged between 9 and 45 years. They were admitted to the burn unit in Mansoura University Hospital after sustaining mixed deep partial and full-thickness flame burn in house fire. Their mean total burned body surface area was 30%. Patients were grafted, on average of 2 weeks of injury, with some as late as 37 days.

*Application of AlloDerm to wounds:*

The AlloDerm package was opened in the operating room, the freez-dried dermal matrix was washed twice in a minimum of 100 ml normal saline solution or lactated ringer's solution at room temperature and then rehydrated in at least 100 ml of the same solution for at least 20 minutes before application (Fig. 1). Each wound site was excised to subcutaneous fat, or fascia (Fig. 2). Haemostasis was achieved with electrocoagulation. Following haemostasis, a 10 x 10 cm piece of the rehydrated dermal matrix was meshed but not expanded and applied to the test site (Fig. 3), covered with an ultra thin split-thickness autograft (range, 0.006 to 0.008 inch) (Fig. 4) and affixed to the wound margins with staples or stitches. The control site received an ultra thin split-thickness autograft alone (Fig. 4). Split-thickness skin grafts were harvested with the brown electric dermatome or Watson skin graft knife.

*Dressing of wounds:*

Dress the wounds with a primary layer of non-adherent petrolatum or ointment impregnated gauze, covered by damp saline-solution gauze and an outer-pressure dressing. Antibiotics in the ointment or the saline-solution were optimal. The primary inner-dressing layer (against the graft) was not to be changed until day 5 to day 7. The outer layers could be changed as early as day 1.

*Evaluation of wounds:*

Wounds were examined and photographed on days 7 and 14 after grafting and at intervals thereafter. Intervals for subsequent formal wound evaluations were 4, 12 and 24 weeks after grafting. Wounds were scored for the percentage of "take" of the overlying ultra thin skin graft and for the following clinical parameters: (1) cosmetic appearance; (2) elasticity; (3) smoothness (4) elevation; (5) durability. Donor site was also evaluated. Cosmesis was evaluated by determining if the two sites were equal or one was either slightly or significantly better than the other. Elasticity was assessed by comparing each site with normal skin using the response to digital pressure and pinching between the thumb and index finger as the measured parameters and expressing this as a percentage of normal. Other characteristics observed were colour, smoothness, elevation and durability.

*Histological evaluation:*

Histological and immunohistochemical examination were performed on AlloDerm material to evaluate its morphological structure. Samples were taken from both AlloDerm after its rehydration during operation and control skin (autograft). They were divided into 2 portions. The first portion was fixed in 10% neutral buffered formalin. Seven-micron sections were cut from paraffin-embedded tissue and stained with haematoxylin and eosin (Hx & E) and Verhoeff-Van Gieson's stain [18] to determine the distribution of collagen and elastin fibers in Al-

loDerm material compared to control skin. The second part of the specimens was cryoprotected in 20% sucrose and frozen in a low-temperature embedding medium. Seven micron Cryo-cut sections were obtained and mounted on clearn coated-glass slides and stained immunohistochemically with monoclonal antibodies directed against type IV collagen (Clon No Col-94, 1926), type VII collagen (Colon no LH, 2.7, Cb 805), Laminin (Clon no LAM-89, L8271), major histocompatibility complex (MHC) class I (clon no W6132, H1650) and class II (clon no HK19, C0817) (Sigma, St. Louis, Mo) using indirect peroxidase conjugate procedure of Nakane [**19**]. After that, a peroxidase-conjugated goat anti-mouse IgG was added. Antibody binding was visualized with amino-ethyl carbazol hydrochloride (AEC) staining kit (Sigma) to give the colored end product. The sections were counter-stained with Mayer's Hx and cover slip while still wet in glycerol gelatin. The slides of control skin (autograft) were served as positive control. Negative control slides were put under the same conditions after omitting primary antibodies or incubated with phosphate buffered saline only.

Tissue biopsies were obtained from Allograft dermal matrix (test sites) and control (autograft) wounds at postoperative weeks 2, 4, 12, 24 respectively. Paraffin sections were prepared and stained with Hx & E and Verhoeff-Van Gieson's stain to identify the persistence of AlloDerm matrix constituents (collagen-elastin) and the progress of healing process after grafting.

## RESULTS

*Clinical evaluation:*

The mean "take" at 14 days after surgery was 98% for dermal matrix, 97.7% for the thin overlying autograft and 99.5% for the control site (Table 1). The differences in "take" did not reach statistical significance for any of the conditions because of the low number of patients in each group.

The comparison of the cosmotic appearance and elasticity of the test and control sites was performed by both the patient and the treating surgeon (Table 2).

As compared to the control site, the elasticity of the site receiving AlloDerm was equivalent after 4 weeks of grafting. However, after 8 weeks the control site developed occasional traumatic blistering whereas the site receiving AlloDerm did not. Moreover, after 12 weeks the site receiving AlloDerm showed increased elasticity, a smoother, less elevated surfaces and overall improved cosmesis (Fig. 5).

The donor site where the ultra-thin autograft was harvested for use over the AlloDerm graft, was scarcely visible. The area showed no elevation, had good skin colour and no discomfort. Cosmetic appearance of the ultra-thin donor sites was determined excellent by both the physician and the patient (Fig. 6).

*Histological evaluation:*

Histological staining of the AlloDerm material with Hx & E and Verhoeff-Van Gieson's stains before grafting showed the normal distribution of collagen and elastin fibres throughout the dermis compared to control skin. However, there was no epidermis or cellular material in the AlloDerm (Figs. 7 & 8).

Immunohistochemical staining of AlloDerm material before grafting showed the morphological structure of the BMC and the extracellular matrix component. The BMC contained type IV collagen which revealed positive staining at the dermal-epidermal junction and at the dermal blood vessels (Fig. 9). It also contained highly specialized type VII collagen (Fig. 10). Laminin was already present at the BMC at the dermal-epidermal junction and was associated with dermal vascular structures (Fig. 11). The staining for MHC class I and class II revealed that the control human skin was immunoperoxidase positive for MHC class I antigen on keratinocytes, dermal endothelial cells and fibroblasts. However, there was a complete absence of staining on AlloDerm (Fig. 12). As regard to MHC class II antigen, an immunoperoxidase positive staining was observed on Langerhans cells of the epidermis and the endothelial cells of the dermis while the sections of AlloDerm were negatively stained (Fig. 13).

Two weeks after grafting the specimens showed re-epithelization of the entire basement membrane of the AlloDerm. The migrating keratinocytes formed a multilayered epidermis with early keratinization. There was substantial ingrowth of fibroblasts and the inflammatory cell infiltrate was not observed within the dermal matrix. There was also substantial dermal

revascularization that reappeared to follow the original vascular channels in the dermal matrix (Fig. 14).

Specimens obtained at postoperative week 4 showed normal distribution of collagen and elastin fibers in dermal matrix. After 12 weeks of grafting fine elastic fibers typical of papillary dermis appeared at the site that were grafted with AlloDerm. However, the elastin fibers were absent at the autografted (control) sites (Fig. 15). By the end of week 24 after grafting, the biopsies revealed a flat dermal-epidermal junction and disorganized dermal architecture at the autografted sites. In contrast, the sites grafted with allograft dermal matrix exhibited the dermal-epidermal undulations and dermal architecture typical of papillary dermis (Fig. 16).

Table (1): Take rates.

| Site | Treatment | Mean "take" rate % |
|---|---|---|
| Control | Ultra thin split-thickness skin graft | 99.5% |
| Test | Dermal matrix | 98% |
| | Ultra thin STSG over dermal matrix | 97.7% |

Table (2): Long-term clinical profile of graft sites.

| Parameters | Test (*t*) / control (C) | | |
|---|---|---|---|
| | 4 weeks | 8 weeks | 12 weeks |
| Elasticity (% normal) # | 30%/30% | 60%/30% | 75%/0% |
| Cosmesis (patient) | T=C | T > C | T > C |
| Cosmesis (Surgeon)* | F/F | F/F | G/P |

# Elasticity is measured as normal, where normal is the elasticity of an adjacent section of undamaged skin.
* Sugeon's assessment of cosmesis was rated as poor (P), fair (F), good (G).

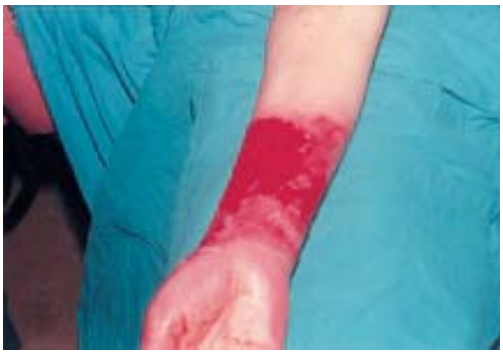

Fig. (1): Rehydration of AlloDerm in normal saline or lactated ringer's before application.

Fig. (2): Deep burn wound was debrided and haemostasis established prior to grafting.

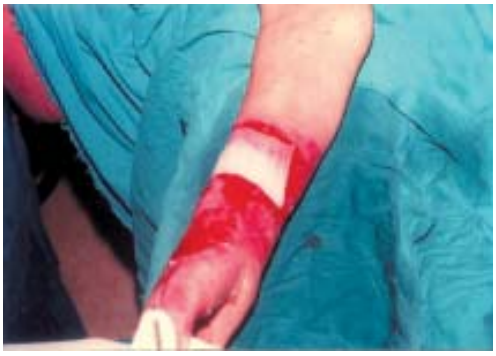

Fig. (3): Application of AlloDerm graft to test site.

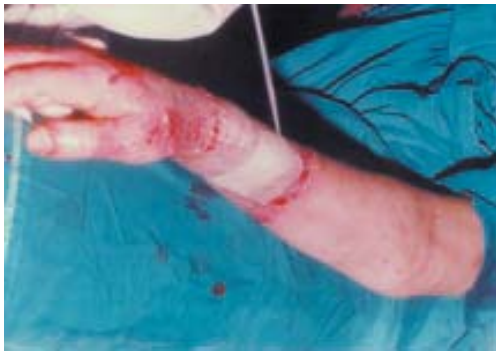

Fig. (4): Application of an ultra-thin autograft over AlloDerm and over control site.

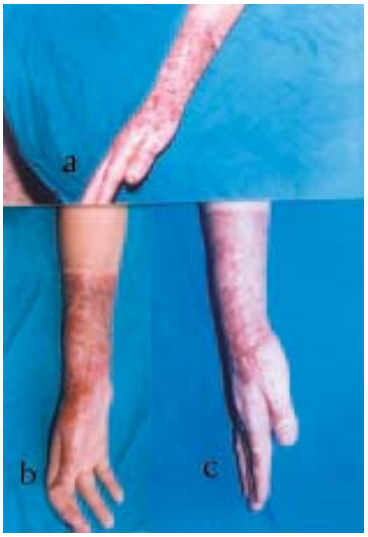


Fig. (5): Test and control sites at days 7 (a), 21 (b) and 120 (c).

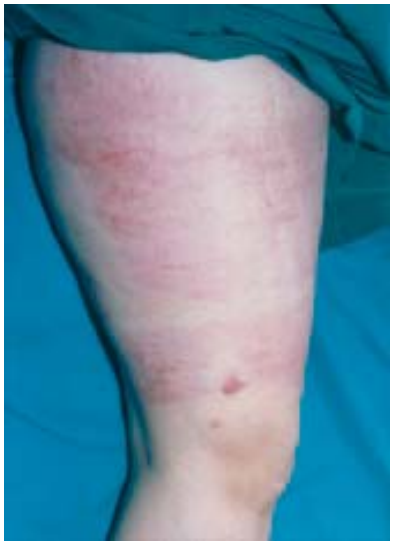

Fig. (6): Ultra-thin donor site 15 days postoperative.

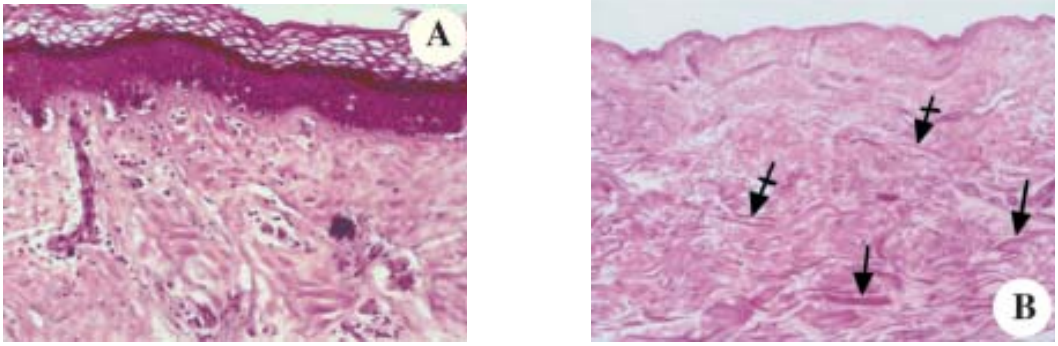


Fig. (7): A photomicrograph of paraffin sections of (A) control human skin and (B) AlloDerm material. Note the absence of cellular material and the epidermis from the AlloDerm and the maintenance of extracellular matrix structural integrity as collagen (arrows) and elastin fibers (crossed arrows) (Hx & E X 250).

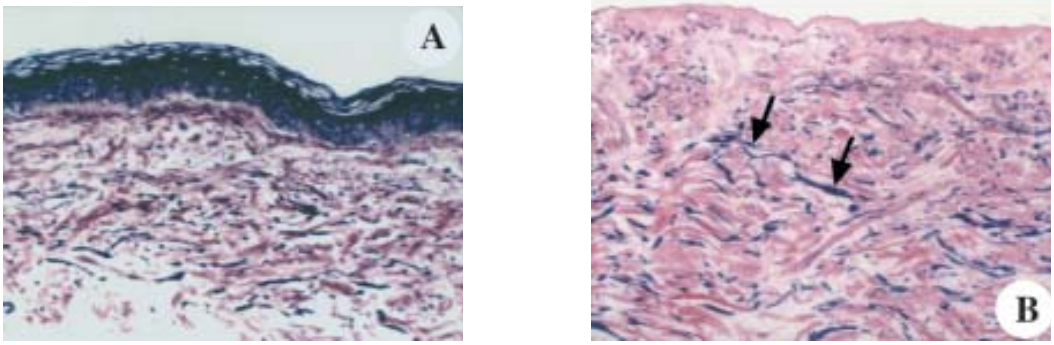


Fig. (8): A photomicrograph of paraffin sections of (A) control human skin and (B) AlloDerm material. The elastin fibres stain dark blue (arrows) and persist in a normal papillary pattern in the AlloDerm (Verhoeff-Van Gieson X 250).

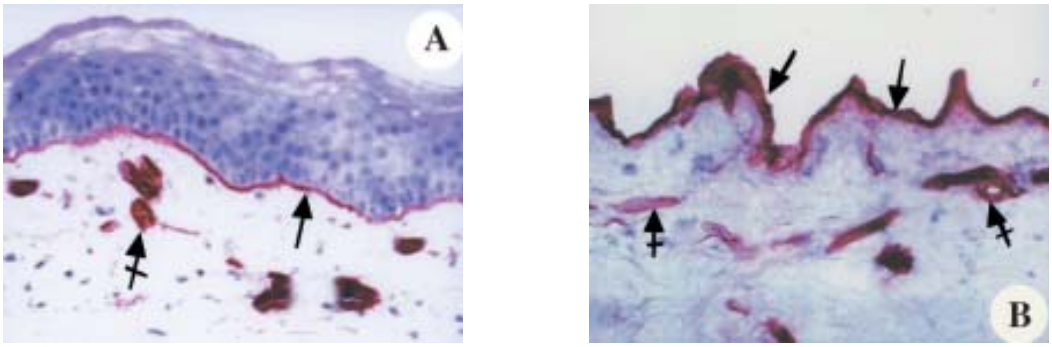


Fig. (9): A photomicrograph of frozen sections of (A) control human skin and (B) AlloDerm material showing immunoperoxidase positive staining for type IV collagen at the BMC of both dermal-epidermal junction (arrows) and dermal blood vessels (crossed arrows) (Immunoperoxidase X 400).

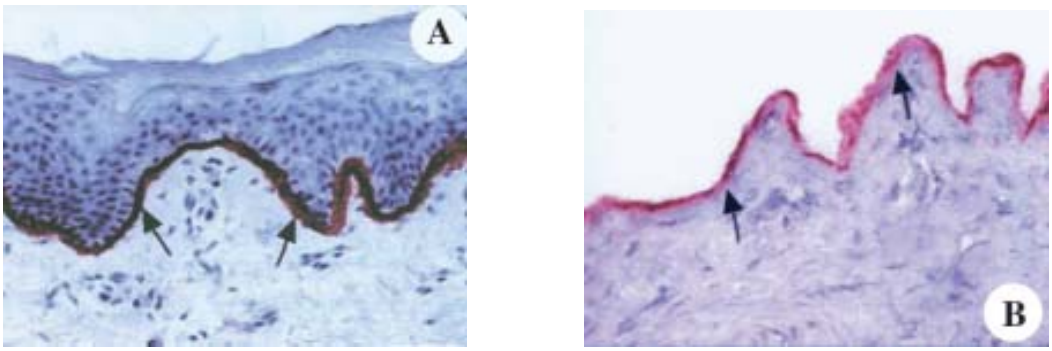


Fig. (10): A photomicrogaph of frozen sections of (A) control human skin and (B) AlloDerm material showing immunoperoxidase positive staining for type VII collagen which is restricted to the BMC of the dermal-epidermal junction (arrows) of both control and AlloDerm material (Immunoperoxidase X 400).

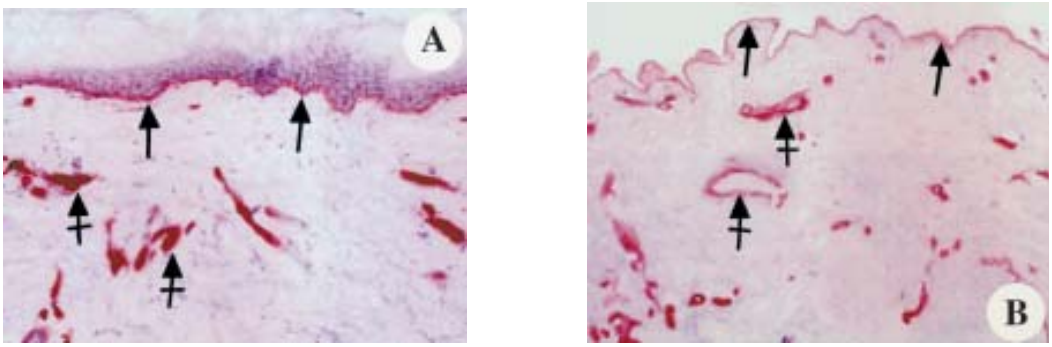


Fig. (11): A photomicrograph of frozen sections of (A) control human skin and (B) AlloDerm material showing immunoperoxidase positive staining for laminin. Note the distribution of the reaction at the BMC of both dermal-epidermal junction (arrows) and the dermal blood vessels (crossed arrows) of both control and AlloDerm material (Immunoperoxidase X 250).

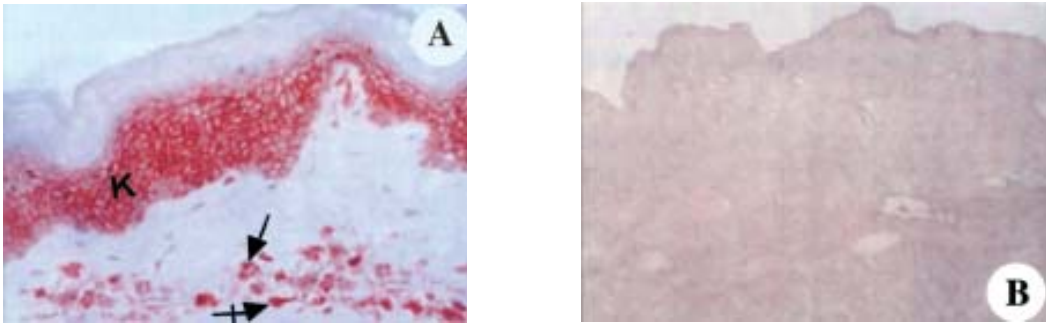


Fig. (12): A photomicrograph of frozen sections of (A) control human skin and (B) AlloDerm material. (A) shows immunoperoxidase positive staining for MHC class I antigen on keratinocytes (K), dermal endothelial cells (arrow) and fibroblasts (crossed arrow). (B) shows complete absence of staining from the AlloDerm (Immunoperoxidase X 400).

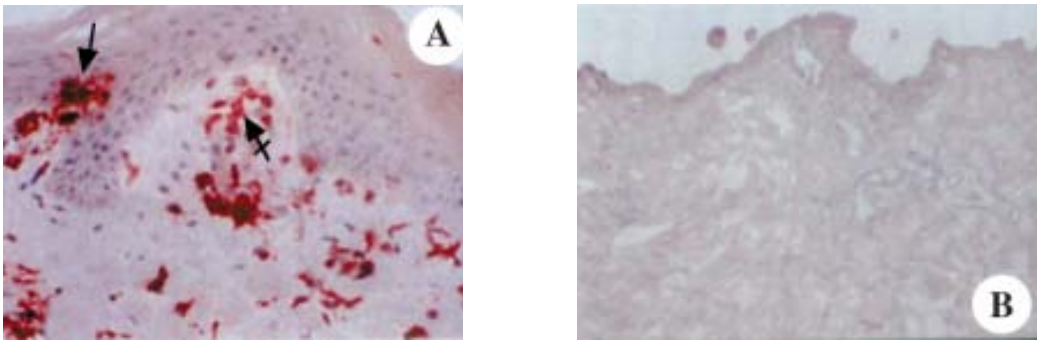


Fig. (13): A phoptomicrograph of frozen sections of (A) control human skin and (B) AlloDerm material. (A) shows immunoperoxidase positive staining for MHC class II antigen on langerhans cells of the epidermis (arrow) and endothelial cells of the dermis (crossed arrow). (B) shows the negative stain of AlloDerm (Immunoperoxidase X 400).

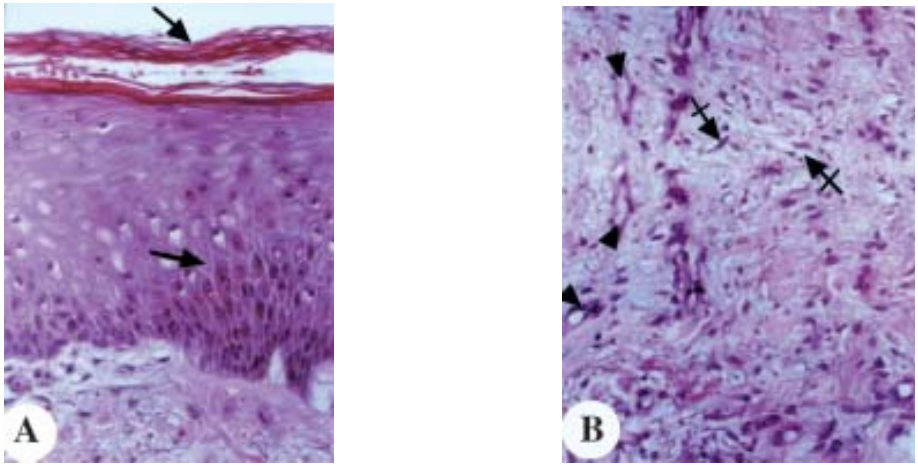


Fig. (14): A photomicrograph of paraffin sections of skin biopsies from AlloDerm covered with ultra thin skin graft 2 weeks after grafting (A) shows multilayered keratinized epidermis (arrows). (B) shows normal fibroblasts repopulation (crossed arrows) and blood capillaries (head arrows) (Hx & E X 400).

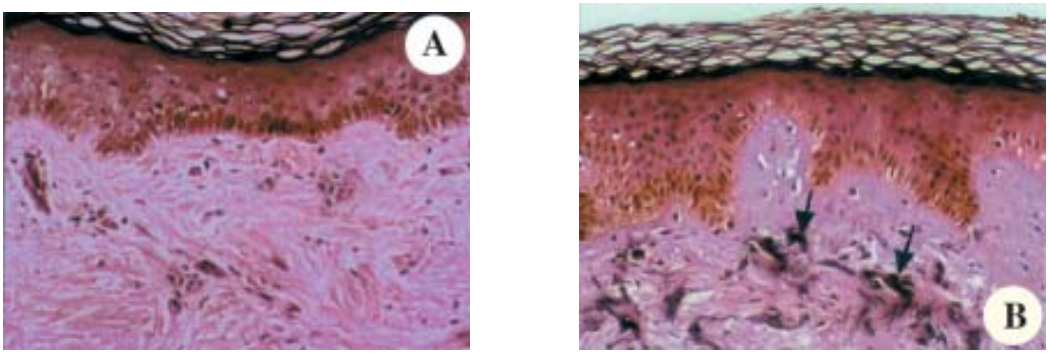


Fig. (15): A photomicrograph of paraffin sections of skin biopsies from (A) autografted (control) site and (B) AlloDerm covered with ultra thin partial-thickness skin graft after 12 weeks of grafting. (A) shows the absence of elastin fibers at the dermis. (B) shows the fine elastic fibers typical of papillary dermis (arrows) (Verhoeff-Van Gieson X 400).

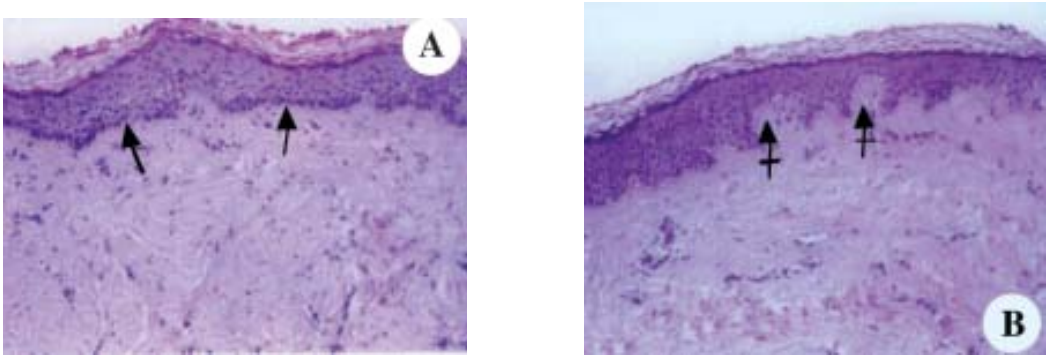


Fig. (16): A photomicrograph of paraffin sections of skin biopsies from (A) autografted (control) skin and (B) AlloDerm covered with ultra thin partial-thickness skin graft after 24 weeks of grafting. (A) shows a flat dermal-epidermal junction (arrow). (B) shows the dermal-epidermal undulations (crossed arrows) and dermal architecture typical of papillary dermis (Hx & E X 160).

## DISCUSSION

Surgeons are well acquainted with the fact that thick split-thickness autografts yield the best cosmetic results in burn victims. However, there is significant donor site trauma associated with thick autografts. This trauma translates into delayed donor site healing, longer periods of time between recroppings, scar formation or altered pigmentation at the donor site, longer hospitalization and higher risk of donor site infection. With greater number of patients now surviving significant burn injuries, the burn surgeon's focus is now directed toward maintaining or improving the functional and cosmetic quality of burn wound healing while simultaneously reducing donor site trauma.

Attempts to restore the critical function of dermis while minimizing donor site trauma have led to the recent development of several biosynthetic dermal analogs. These analogs are typically composed of neonatal fibroblasts, cultured in sheets of a woven material such as polyglycolic acid, bovine collagen gel or cross-linked collagen-glycosaminoglycan matrix [**20-22**]. These biosynthetic dermal substitutes have been widely studied in animal models, but have gained little accepted as dermal substitutes in the treatment of full-thickness burns. The role of these dermal substitutes was based on the premise that the matrix or mesh supporting the cultured fibroblasts will degraded in the wound healing process, leaving behind the fibroblasts and their secreted dermal proteins [**23**]. It was reported, that only fetal fibroblasts in utero are capable of regenerating dermal extracellular matrix without scarring [**24**]. However, mature fibroblasts repair damaged dermis through the formation of granulation tissue, which matures to scar tissue.

The observation that mature fibroblasts do not regenerate the complex architecture of dermis, focused attention on the matrix deficit in full-thickness injury and prompted the development of a process to generate a structurally and biochemically intact acellular allograft dermal matrix (AlloDerm), intended to function as a template for dermal regeneration **[25,26]**.

In the present work, the morphological aspect of the AlloDerm material showed the characteric normal pattern of collagen and elastin bundles. Immunohistochemally, the BMC at the dermal-epidermal junction was intact and contained type IV collagen, Laminin and highly specialized type VII collagen. The BMC of the allograft dermal matrix supported the migration and attachment of keratinocytes from the overlying split-thickness autograft. The importance of basement membrane integrity in the normal process of wound healing has been reported by Chetty **[27]**, who demonstrated that the BMC under blisters in burn patients receiving meshed grafts was reduced and/or irregular. Another evidence of the importance of a continuous BMC comes from previous clinical trials done by Compton and his colleagues **[28]**, in which cultured keratinocytes sheets were grafted onto full-thickness burn wounds. These grafts resulted in the formation of fragile skin and blistering problems. In the current study, histological analysis showed that the keratinocytes preferentially attached to the BMC of the grafted matrix, 2 weeks post surgery. This indicates that the meshed autograft functioned primarily as a carrier or delivery vehicle for keratinocytes and the BMC is important for attachment and differentiation of epidermal grafts. The attachment of keratinocytes is dependant on retention of Laminin and type IV collagen in the BMC as they ensure strong physical bonding of epidermis to dermis **[29,30]**. Laminin has been considered as the major non collagenous component of the BMC. It is important for cell attachment and maintainence of the differentiated state of epithelial and endothelial layers that are intimately associated with the BMC **[31,32]**.

The normal collagen bundle pattern and the presence of elastin were evident in the biopsy at week 4 postsurgery. These results correlate with the clinical observations of a durable wound cover without blistering or fragility. Since AlloDerm dermal grafts contained elastic fibers and the turnover of elastin is approximately 2-3 years **[28]**, this component of the Alloderm-dermal graft has persisted in the graft site and absent from control site. The preservation of these dermal component explain the superiority of elasticity and cosmesis of the dermal matrix when compared to the control site.

Collagen fibers in scar tissue are oriented in dense bundles parallel to the lines of stress while elastin is typically absent **[33]**. Abnormal orientation of collagen and the absence of elastin in scar tissue, are causative factors in scar contracture **[34]**. However, our results revealed that the dermal architecture has been maintained during tissue remodeling with more randomized collagen bundle organization. This picture might prevent scar formation **[35,36]**.

It has been reported that the immunogenicity of the epidermis is high whereas that of the dermis is low **[13]**. This has led to the belief that allograft dermis can be transplanted without rejection **[37]**. Attempts have been made to clinically incorporate allogenic dermis into full thickness wounds. Yang **[38]**, grafted allograft sheets containing holes that were then filled with autograft plugs. With time, the allograft epidermis sloughed and the remaining component of the allograft dermis was covered with migrating epidermis from the autograft plugs.

Although the majority of immunogenic cells are found in the epidermis, a number of dermal cells also express MHC antigens. These cells include dendritic cells, endothelial cells and in the presence of interferon, fibroblasts **[39,40]**. Endothelial cells in particular express and can present MHC class II antigens and are the first contact between immunocompetent cells of the host and the graft **[41]**. As such, they are the primary site of the specific immune rejection response to allograft skin **[42]**. The resulting perivascular inflammation in the dermis causes sloughing of the epidermis secondary to hypoxia. The present work showed that the AlloDerm process was developed to remove the cells of both the epidermis and dermis while maintaining the structural and biochemical integrity of the dermal extracellular matrix and the BMC. By removing all cells of the epidermis and the dermis, the specific immune response was avoided.

Scaring and contracture resulting from full-thickness skin injury are inversely proportional to the amount of dermis transferred in a con-

ventional split thickness skin graft [43]. However, the current study revealed that a grafted acellular dermal matrix will support fibroblast infiltration that maintained their capacity to produce a more mature dermis. There was also new vascularization and epithelization. Furthermore, the absence of an inflammatory cell infiltrate after two weeks of grafting suggested that allograft matrix was non immunogenic. Electron microscopic studies showed that there was no myofibroblast in allograft dermal matrix. Apparently, the dermal substitute reduced the differentiation of firboblasts into myofibroblasts. This effect might also be mediated by cytokines able to bind to collagen-elastin dermal substitute [44].

In the present study, one of the most important observations was the higher "take" rates (97.7%) of thinner autografts that probably reflect the unique physiology of the allograft dermal matrix site. The autograft is separated from the vascular bed by the initially avascular dermal matrix. Cellular elements of the autografts are nourished by serum through the interstices of the meshed allograft dermal matrix. The less metabolic demands and the shorter distance that nutrients travel to reach the epidermal cells improved the take of thinner autograft.

Donor sites are a morbidity. They are painful, can become hypertrophic, hyperpigmented, hypopigmented, erythematous and thickened. They ultimately may have a worse cosmetic appearance than the grafted wound. Also, delayed healing of donor sites can be costly and life threatening, especially in pediatric or elderly patients and those with large total body surface area burns. Very often patients are discharged early and have to care for the wound by themselves. The sooner the donor site heals, the sooner you can eliminate all of these potential problems. If you have a thin donor site, the type used in this study with AlloDerm, that is certainly going to heal faster, less depigmentation and reduced hypertrophic scarring. We found that AlloDerm helps decrease morbidity and decreases the risk of infection as the time of healing is shorter. Thin split-thickness autografts, are typically associated with a higher incidence of burn wound scarring and contracture, as a result of the minimal amount of dermis provided [45]. In the present work, histological analysis of test site biopsy supported the conclusion that the dermal matrix was additive to the overlying autograft epidermis.

In this study, it was found that there was faster maturity of the wound with AlloDerm, with less contracture and better function. If you can obviate further reconstructive surgeries and get the patients functioning with less deformity, then that is to everyone's benefit. It is also more cost-effective. If you can get patients back to work faster, that is very cost-effective, because you have to figure in the loss of wages as part of the cost of the injury.

In conclusion, with AlloDerm, burn care specialists now have an alternative to conventional autografting. AlloDerm acellular dermal graft serves as a dermal transplant for deep-partial and full-thickness burns. With AlloDerm, only ultra-thin epithelial autografts are required from the patient. The use of ultra-thin autografts result in faster healing of donor sites, fewer donor site morbidity and reduced hospitalization costs.

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