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Original Research Article.

Assessment of Biological Response of Cadaver Dermal Allograft in Burn and Non Healing Ulcer Wounds

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ABSTRACT

Objectives: The purpose of the study was to place cadaver dermal allograft in patients with burn and non-healing ulcer wounds and observe their biologic response.

1. To identify clinical success rate of take of cadaveric dermal allograft
2. To evaluate infection / rejection rate of cadaveric dermal allograft
3. To confirm take of allograft by histological features and neovascularization after applying cadaveric dermal allograft.

Method: This study was done on 50 patients suffering from burn and nonhealing ulcer wounds. Patients of all age groups and both sexes were taken. Dermal allografts were harvested from cadaver after taking consent from relatives. These grafts were applied to wound sites after treating it with glycerol and cryopreservation and their biological responses were assessed.

Results: In this study, on follow up for 2 months, out of 50 patients, the graft was survived in 41 (82%) patients and rejected in 9 (18%) patients. Out of 41 patients, the graft was detached and wounds were completely healed in 33(66%) patients and graft was still intact in 8(16%) patients. Graft take up success was assessed by histopathologically showing neovascularization.

Conclusion: It was observed that epidermis depleted allograft has proved to be an effective material for wound coverage due to absence of langerhans cells. Further cryopreservation increased its viability and reduced immune reaction. Glycerol acted as an effective virucidal agent.

Keywords: Dermal Allograft, Cryopreservation, Neovascularisation.

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INTRODUCTION

The ideal skin replacement after thermal injury i.e. skin auto graft, consist of epidermis integrated by means of basement membrane zone to dermis. In patient with massive burn injuries and limited skin donor site, long terms problems of skin loss must be solved by use of alternative wound closure materials.

In patients with massive burn injuries, temporary wound coverage is required when there are limited autologous donor sites. The use of allograft donor skin as a permanent skin transplant in full thickness burns is limited by its immunogenic properties. Allograft skin will initially take on a full thickness wound but is ultimately rejected. This immunogenic response to allograft skin is directed primarily against the langerhans cells of epidermis. Therefore, the cryopreserved dermal allograft from cadaver source has proved to be an excellent material for burn and non-healing ulcer wounds.[1-3]

MATERIALS AND METHODS

In this study evaluation of state of wound healing has been done in the form of cryopreserved cadaver dermal allograft. The study was designed as a prospective clinical trial conducted in Santosh Medical College, Ghaziabad, U.P.

Fifty patients with second and third degree burns and non-healing ulcers were included in this study irrespective of age, sex and nutritional status. Written informed consent was taken from the recipient of the graft.

Technique of Harvesting Dermal Allograft

Skin graft was harvested from cadavers with negative viral markers, within 24 hours after death and certain exclusion criteria were followed:

Those patients who died with

1. Malignancy
2. Jaundice
3. Sepsis
4. Poisoning

Skin grafts were harvested with usual operating room technique. The donor skin was prepared using betadine and conventional sterile gowns, gloves and instruments were used. Skin grafts were taken from thigh, arm and forearm.

Immediately following harvesting, grafts were immersed in 15% glycerol solution with Ringer's lactate solution for a period of two hour at 4° C.

Freezing and Banking: After 2 hour skin graft sheets were laid on aseptic wet gauge piece and packed in pre sterilized polythene bags. The plastic bags were kept in deep freezer.

Operative Procedures

Recipient site had been prepared by regular dressing till healthy granulation tissue had appeared on the bed. All patients were given preoperative antibiotics. The grafting was done under full aseptic precautions.

After cleaning the wound with betadine thoroughly, under local anesthesia, grafts were applied and stitched with the suture material (chromic 2-0).The grafted area was thoroughly irrigated with betadine. The wound was dressed with sofratulle and betadine soaked gauge pieces and tight bandage was applied.

Wounds, after grafting, were seen clinically on 5th, 7th, 10th and 20th post-operative days.

The clinical observations of take were confirmed on 14th day with histological evaluation of biopsy for host cell infiltration and neovascularization of applied dermal allograft.

When required pus was sent for culture and sensitivity report to detect micro-organism and to detect infection rate so that appropriate antibiotic could be used.

OBSERVATIONS AND RESULTS

The evaluation of state of wound healing in the form of cryopreserved cadaveric dermal allografting was done.

There were 50 patients who were studied for the evaluation of state of wound healing. Out of which 32(64%) were male and 18 (36%) were female. Out of them thermal burn patients were 22(44%), 4 (8%) patients of electric burn, 17(34%) patients of non-healing ulcers, 1(2%) patient of Marjolin's ulcer, and 6 (12%) patients of degloving injury. (Table 1)

In this study cryopreserved cadaver dermal grafts were applied on the different sites of the body. Out of 50 patients, cadaveric dermal grafting was done on the chest in 3(6%) patients, on back in 1(2%) patient, on abdomen in 1(2%) patient, on arm in 1 (2%) patient, on forearm in 6 (12%) patients, on thigh in 15(30%) patients, on leg in 9 (18%) patients and on foot in 14 (28%) patients.

The biological assessment of cryopreserved dermal allografts by clinical evaluation was done on 5th, 7th, 10th and 20th post-operative days after application of grafts on the basic color, swelling, exudation, texture and complications.

1. **COLOUR:** On observation the 7th post-operative day all grafts ware pale in color, suggesting ischemia and avascularity. On 10th post-operative day, out of 50 patients the color of dermal allograft turned to pink in 38 (76%) patients suggesting neovascularization. On 20th post-operative day the color of dermal allograft remained pink in 41 (82%) patients.
2. **SWELLING:** The swelling on grafted areas was assessed and till 7th post-operative day, swelling was not found on grafted area. On the 10th post-operative day, out of 50 patients swelling was found in 38 (76%) patients suggesting inflammatory process. On 20th post-operative day the swelling on grafted area was found in 41(82%) patients.
3. **EXUDATION:** Till 7th post-operative day no exudates was seen on grafted area. On 10th post-operative day, out of 50 patients serous exudation was seen in 38 (76%) patients suggesting inflammatory process. On 20th post-operative day serous exudation was seen in 41(82%) patients.
4. **CONSISTENCY:** The consistency of applied dermal graft was assessed by palpation. On 5th post-operative day almost all dermal allografts were soft in consistency. On the 7th post-operative day consistency of 46 (92%) grafts remained soft, while in 4(8%) patients consistency became hard. On 10th post-operative day the consistency was still soft in 40 (80%) patients where as in 6(12%) patients consistency changed to slightly hard to leathery texture while in remaining 4 patients, grafts were totally sloughed off and rejected. On 20th post-operative day, the consistency remained hard in 3(6%) patients, whereas, 5 grafts were rejected. consistency of graft was still soft in 38(76%) patients.

Table 1: Distribution of Patients According to Diagnosis

No	Diagnosis	Male	Female	Total
1.	Thermal burn	11	11	22
2.	Electric burn	3	1	4
3.	Non healing ulcer	12	5	17
4.	Degloving injury	5	1	6
5.	Marjolin's ulcer	1	0	1
	Total	32	18	50

Table 2: Number of Patients Showing Color of Graft

	Post-operative day			
Color	5th	7th	10th	20th
Pink	-	-	38(76%)	41(82%)
Pale	50(100%)	50(100%)	12(24%)	9(18%)

Table 3: Number of Patients Showing Swelling of Graft

	Post-operative day			
Swelling	5th	7th	10th	20th
Present	-	-	38(76%)	41(82%)
Absent	50(100%)	50(100%)	12(24%)	9(18%)

Table 4: Number of Patients Showing Exudation on Graft

	Post-operative day			
Exudation	5th	7th	10th	20th
Present	-	-	38(76%)	41(82%)
Absent	50(100%)	50(100%)	12(24%)	9(18%)

Table 5: Number of Patients Showing Consistency of Graft

	Post-operative day			
Consistency	5th	7th	10th	20th
Soft	50(100%)	46(92%)	40(80%)	38(76%)
Hard	-	4(8%)	6(12%)	3(6%)

Table 6: Number of Patients Showing Complications

Complications	No. of Patients
No Complication	17(34%)
Necrosis From Margin	15(30%)
Necrosis In Center	9(18%)
Completely Infected And Necrosed	9(18%)

Table 7: Number of Patients Showing Take of Grafts

Take of Grafts	No. of Patients
>90%.	17(34%)
80-90%.	14 (28%)
70-79%	4 (8%)
60-69%.	4 (8%)
<60%.	2 (4%)
Rejected	9 (18%)

Table 8: Number of Patients Showing Bacterial Contamination

Bacterial Contamination	No. of Patients
Staphylococcus	6(12%)
E. Coli	4(8%)
Pseudomonas	5(10%)
Other (Klebsiella, streptococcus)	3 (6%)
Sterile	32(64%)

5. **COMPLICATION:** All patients were observed approximately after one month for complication i.e. contraction of dermal allograft, infection and necrosis. The following observations were noted. The observed grafts were healthy in 17 (34%) patients at the end of approximately one month. In 15 (30%) patients grafts were necrosed from margin and in 9 (18%) patients graft were necrosed in center. While in 9(18%) patients graft became completely infected and necrosis of whole graft occurred.
6. **VIABILITY:** At the end of one month in 17(34%) patients the take of graft was >90%. In 14 (28%) patients the take of graft was 80-90%. In 4 (8%) patients the take of graft was 70-79%. In 4 (8%) patients the take of graft was 60-69%. In 2 (4%) patients the take of graft was <60%. In 9 (18%) patients graft was totally sloughed off & detached from their bed. Out of 9 patients, 4(8%) patients graft were rejected on 10th P.O. day and 5 (10%) patients graft were rejected on 20th P.O day.
7. **LONG TERM FOLLOW UP:** All 41 (82%) patients in which graft survived were followed up for approximately 2 months. Out of 41 (82%) patients, in 33(66%) patients graft was detached from their bed & wounds were completely healed. In 8 (16%) patients graft was still intact with their bed.
8. **HISTOPATHOLOGY:** Histopathological observation was done on 14th post-operative day. In cryopreserved cadaver dermal allografts on 14th day, the superficial dermis showed infiltration of mononuclear cells. Keratinization was also seen. In cryopreserved allografts angiogenesis was seen at the junction of graft and graft bed. These graft also showed acanthosis and spongiosis however these were not sloughed off. Degenerative changes in vessels might be attributed for commencement of process of rejection of allografts. Proliferation of fibroblast and synthesis of collagen during initial stage of healing raised the level of collagen on 14th day. The level of collagen in healing tissue was directly correlated with the fibroblasts. On 14th day in cryopreserved allografts, collagen bundles were uniformly arranged in the graft. The grafts which were going to be rejected showed irregularly arranged collagen bundles and degenerative changes of vascular wall in dermis, which reflected commencement of rejection process. Granulation tissue along with infiltration of mononuclear cells was evident on graft bed.
9. **MICROBIOLOGICAL STUDIES:** Few microorganisms were identified with the help of pus culture reports. Out of 50 patients, 18 (36%) patient showed bacterial growth in pus culture while in 32(64%) patient pus culture was sterile. Staphylococcus was found in 6(12%) patients. E. Coli was found in 4(8%) patients. Pseudomonas was found in 5(10%) patients. Other (Klebsiella, streptococcus) were also found in 3 (6%) patients

DISCUSSION

In the present study, it was seen that glycerol treated cryopreserved dermal cadaver allografting has proved to be effective as both mechanical and physiological barrier and its use decreased the loss of water, protein and heat through burn wound and prevented contamination of wound by environmental microorganism.

Epidermal allograft has langerhan's cells which has immunogenic properties and is responsible for alloaggression and ultimately rejection of allografts. This immunogenic property is reduced by cryopreservation.

Further cryopreservation also increases the power of skin to withstand infection by microorganisms which are also responsible for sloughing and rejection of grafts.

Glycerol treatment of dermal allografts is also helpful in reducing cytotoxic T-cell mediated graft rejection process. Further glycerol has been shown to have better cryoprotective activity and it is also helpful in reducing activity of intracellular viruses.

Cell metabolism is reduced by cryopreservation and the ideal medium in which skin is stored should supply electrolytes and nutrients in physiologic concentration.[4,5]

"Herndon" and "Livesey" used porcine skin to produce an acellular dermal matrix and to reduce immunogenic response of the cells of epidermis. They investigated the potential use of this matrix in combination with a meshed split thickness autograft as a permanent allograft in full thickness wounds in pigs. Histological analysis revealed that the dermal matrix supported fibroblast infiltration, neovascularization and keratinocyte migration from an overlying STSG.[6]

"Livesey" used porcine skin and processed it to produce dermal matrix was de- epidermised. He showed fibroblast infiltration, neovascularization and keratinocytes migration, there was no evidence of cell mediated immune response.[2]

"Lattari and Jones" studied use of allograft dermis obtained from human cadaver skin and processed to remove the epidermal fibroblast and endothelial cells that are targets for immune response. He noted rapid revascularization of acellular dermal matrix from wound bed.[7]

"Rennekampff" studied the use of human keratinocytes cultured onto a synthetic hydrophilic dressing. This was applied with acellular dermal matrix in full thickness skin defects. He noted

reepithelialization of wound and pink appearance of wound surface with well-established vascularization in wound after 21 days.[8]

"Purdue" and "John L.Hunt" studied the use of dermograft-TC and human cadaver allograft in full thickness burn patients. They observed the average amount of sloughing was 3.6% in 5th day and increased to 49.9% on 18th day.[9]

Histomorphological Studies

In this study, it was observed that there was infiltration of mononeuclear cells, and fibroblast and keratinization, angiogenesis, acanthosis, spongiosis with raised level of collagen in all well survived grafts on 14th post-operative day. There were acanthosis, spongiosis and degenerative changes in vascular wall and irregular collagen bundles in all grafts which were rejected.

"Livesey" observed that wound covered with acellular dermal matrix, obtained from porcine skin showed fibroblast infiltration, neovascularization and keratinocytes migration on histological examination.[6] "Rennekampff" observed that on histological examination on 21st day it showed reepithelialization of wound with dermis was observed with a moderate number of stellate shaped fibroblasts and human collagen type IV.[8]

"Kawanami" studied healing process of frozen skin allograft used in treatment of burns. Histopathologically after 3rd week examination the dermal component of allograft were covered by epithelial cells from recipient tissue and were invaded by fibroblasts and capillaries.[10]

"Josef Aubock" observed dense mononuclear dermal infiltration consisting predominately of activated T- cells, vacuolization. He also observed increased cytotoxic T-cells precursors during graft rejection.[11]

Microbiological Studies

In this study, in 32 (64%) patients, the pus culture was sterile. Out of 18 (36%) patients, staphylococcus was found in 6 (12%) patients, E.coli was found in 4 (8%) patients, Pseudomonas was found in 5 (10%) patients & others (klebsiella, streptococcus) were found in 3 (6%) patients.

Glycerol used as cryoprotectant also served as virucidal and antibacterial agent.

"Marshall" and "Ghosh" studied the effect of glycerol on the inactivation of intracellular viruses. He observed that at 4°C, 85% glycerol could not fully inactivate intracellular HSV- 1 or poliovirus even after 4 weeks, 98% glycerol inactivated intracellular HSV-1 after 3 weeks but could not fully inactivate intracellular poliovirus after 4 weeks. At 20°C, 85% glycerol inactivated intracellular HSV-1within one week.[12]

SUMMARY AND CONCLUSION

1. Thus in this study we observed that langerhans cells depleted cryopreserved dermal cadaver allograft acts as a mechanical and physiological barrier when applied on the patients of burn and non-healing ulcers.
2. The cryopreservation helped in prolong viability of grafts and served in minimizing the immunoreactivity and bacterial contamination.
3. Due to absence of langerhans cells it showed minimal immunogenic rejection.
4. It promotes wound healing by the process of neovascularization and development of granulation tissue.
5. Glycerol used as cryoprotectant also served as virucidal agent and reduced viral transmission from cadaver dermal allografts to patients.

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