A COMPARATIVE STUDY OF USING AUTOGRAFT AND ALLOGRAFT TRANSPLANTATION FORRESURFACE OF FULL-THICKNESSCUTANEOUS WOUNDS IN RABBITS

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ABSTRACT

The current studyaimed to compare between two types of skin grafting and denote which is the best based on clinico-histopathological evaluations.

The study was performed on 24 clinically healthy adult rabbits. The animals were allotted randomly into two groups i.e. autologous and allogeneic groups (12 rabbits/ group). Rabbits were premedicated with atropine sulphate then, anaesthetized generally with a combination of ketamine and xylazine hydrochloride. Each rabbit was positioned in sternal recumbency. Square full thickness skin incision approximately(4cmx4 cm) were created in two areas of the back (anterior and posterior sides), (thus the total wounds numberwere 48). Then blunt dissection was performed to remove the skin flaps. In both groups the grafts were overlap around the perimeter of the recipient site and they were fixed to wound margins with sutures of simple interrupted pattern. Aftercompletion the operation, the wounds were covered with an appropriate non-adhesive bandage which change every 24-72 hours and rabbits were administered systemic antibiotics. Skin biopsies ($5mm^3$) were taken at (7,14 and 21) days post-wounding (8wounds/period).

Results reflected signs of mild inflammation (redness and swelling) which were observed immediately after wounding. The healing time in auto-grafts rabbits group was (15 - 17) days with failure of graft in one rabbits. In contrast healing time was relatively longer (19 - 21) days in allogeneic graft group with failure of grafts in two rabbits. Histopathological findings in both groups showed inflammatory cells infiltration in the first week post-wounding. With the advancement of time mature granulation tissues was formed. In conclusion the best result was achieved by autograft type in comparing with allograft type.

INTRODUCTION

The grafts are simple and reliable method of achieving closure of skin defects. Although any open wound can be grafted, the most common indication for skin grafting is to promote healing of a large wound that cannot be closed primarily or heal by secondary intention (1).There are three types of skin grafting, auto-grafts involves the transfer of skin components harvested from an uninvolved anatomic area to a wounded area on the same animal, grafts from another animal of the same species are allografts, and from another species called xeno-grafts (2 and 3).

A free skin graft is a segment of skin that has no vascular supply because it has been detached completely from its donor site and relocated to a wound at another site (4). The graft rapidly attaches to the wound by fibrin and is nourished initially by capillary action into the lumen of the graft's vessels. Within 48 to 72 hours, vessels and fibroblasts from the wound invade the fibrin. The graft is re-vascularized by day 5, and by day 10, and it is attached firmly to wound (5). Normal wound healing occurs in 3 different phases. The first phase involves hemostasis and inflammation, and this is followed by the second (cellular) phase of fibroplasia and proliferation and the third phase of maturation and remodeling(6 and 7).The aim of the presentstudywas to compare between two types of skin grafting and denote which is the best to resurface the wounded skinbased on clinico-histopathological point of view.

MATERIALS AND METHODS

1. Animals

The studywasperformed on 24 clinically healthy adult rabbits (age 8-12 months) and weighing (1.25-1.5) kg. The animals were allotted randomly into two groups i.e., autologous (T) and allogeneic (A) groups (12 rabbits/ group). The animals were housed in an environmentally controlled animal facility for seven days for acclimatization. Prior to the operation, the rabbits were deprived from food and water for 12 hours. The operative field was the back of the rabbit which wasprepped for aseptic surgical incision. Rabbits were premedicated with atropine sulphate in a dose of 1mg/kg and 15 mins., later, general anaesthesia was done with a combination of ketamine hydrochloride 5% at a dose rate of 40 mg/kg and xylazine hydrochloride 2%, at a dose rate of 10 mg/kg (8). All drugs were injected IM., Each rabbit was positioned in sternal recumbency.Square full thickness skin incision approximately(4cmx4 cm) werecreated (fig.1) in two areas of the back (anterior and posterior), (thus the total wounds number were 48). Bleeding was arrested by routine

manner. Then blunt dissection was performed to remove the skin flaps (fig. 2). In both groups the grafts were overlap around the perimeter of the recipient site and they were fixed to wound margins with 4 sutures on each angle with simple interrupted pattern using silk size 3/0 (fig. 3).

In autologous group, the excised anterior flap was implanted on the posterior aspect. The full thickness skin graft was sutured to the wound edges circumferentially with simple interrupted pattern at 3mm apart with non-absorbable suture material (silk size 0/3) (fig. 4) and visversa for the second flap. In allogeneic group and after injection of sodium hydrocortisone in a dose rate of (5 mg / kgB.W.)IM.,daily for one week as immuno-suppressor (9). Two flaps (same size) in each animal were created on the back. After that skin graftwas transferred from donors site of one rabbit to recipients site of another rabbit. Until transplantation, the grafts are put into sterile normal saline. After the operation, the wounds were covered with an appropriate non-adhesive bandage which change every 24-72 hours depending on the amount and character of the exudate, then rabbits were administered systemic antibiotic (pencilline-streptomycine) IM., in a dose rate of 20.000 IUand 10mg/kg respectively for five consecutive days. The skin stitches were taken-off after10 days post-surgery if secondary complications were not encountered.

2.Histopathological evaluations: A ($5mm^3$) skinbiopsies were taken from the wound edges of each rabbit on 7, 14 and 21 days post-wounding for both groups (12 rabbits/group) and (8 wounds/period). Wound biopsies fixed in (10%) neutral formalin solution, and embedded in paraffin, sectioned at (5-6) micron and staining with hematoxylin and eosin (H&E)dye and examine under light microscope (10).

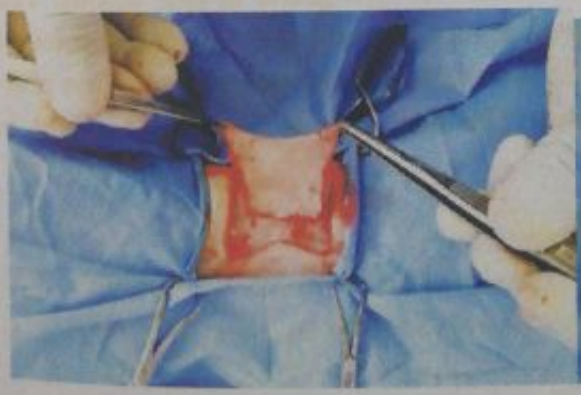

Fig.1. An incision (4x4) cm., is created onthe back of the rabbit and blunt dissection is made to remove the flap.

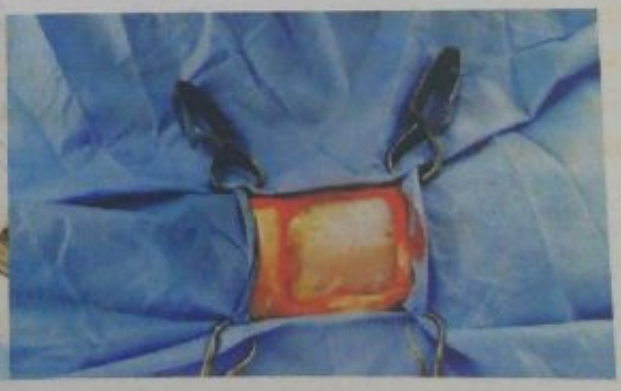

Fig.2. Complete skin flap removal.

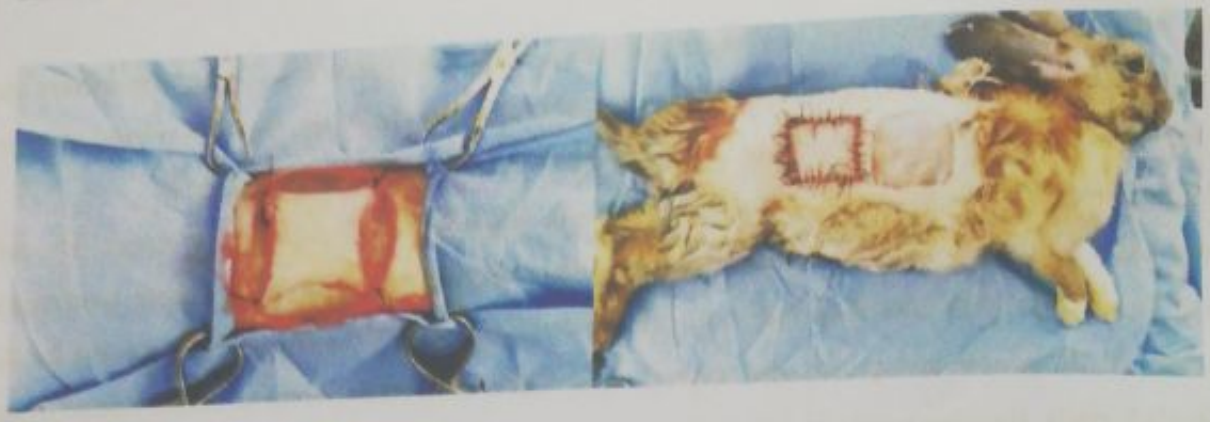

Fig.3.Grafts was overlap around the perimetcrof the recipient site and is fixed towound margins with stitches on eachangle with simple interrupted pattern.

Fig. 4. The anterior flap is implantedon the posterior side and fixed by simple interrupted stitches (silk 0-3).

RESULTS

1. Post-surgical follow-up

Signs of mild inflammation (redness and swelling) were observed immediately after wounding. However, these signs subsided within 3-4 dayspost wounding. None of the wounds displayed edema or exuberant granulation tissue. The healing time of the wound in auto-grafts rabbits group was (15 - 17) days and there was no rejection of auto-grafts in 11out of 12 rabbits (failure of graft). In contrast healing time was relatively longer (19 - 21) days in allogeneic graft group, in addition some grafts showed on the 10 th post-operative day slight hardening in part of grafts borders, reddening specially at the junction between the grafts and the recipient pad and shrinkage of allogeneic graft after15th days which lead to complete failure of two grafts in two rabbits. The remaining rabbits exhibited a firm attachment of the graft at recipient site and complete success of implantation.The hair growth in skin of rabbit with different directions was noticed on14 days post-surgery in auto-grafts groups with less pain response. The hair growth found to be mild in allogeneic group on day 21.

2. The histopathological findings, during different period are illustrated in the following table.

Time (week)	Autograft group (T)	Allogeneic group (A)
One	Neutrophils,mononuclearcells and fibroblasts with hemorrhage in necrotic foreign materials over the incision (fig.5).	Initiated extention of several layers of epithelial cells under neutrophils, macrophages infiltration of the foreign materials with fibroblasts proliferation over granulation tissue (fig.6).
Two	Thin epithelial cellsover granulation tissues consisting from congested blood vesselswith irregular collagen fibers and inflammatary cells infiltration (fig.7).	Several layers thickness epithelial cells extended under inflammatory and cellular debris(fig.8).
Three	Thickness layers of epithelial cells extended under inflammatory and cellular debris and over mature granulation tissues (fig.9).	Epidermal layer over mature granulation tissues(fig.10).

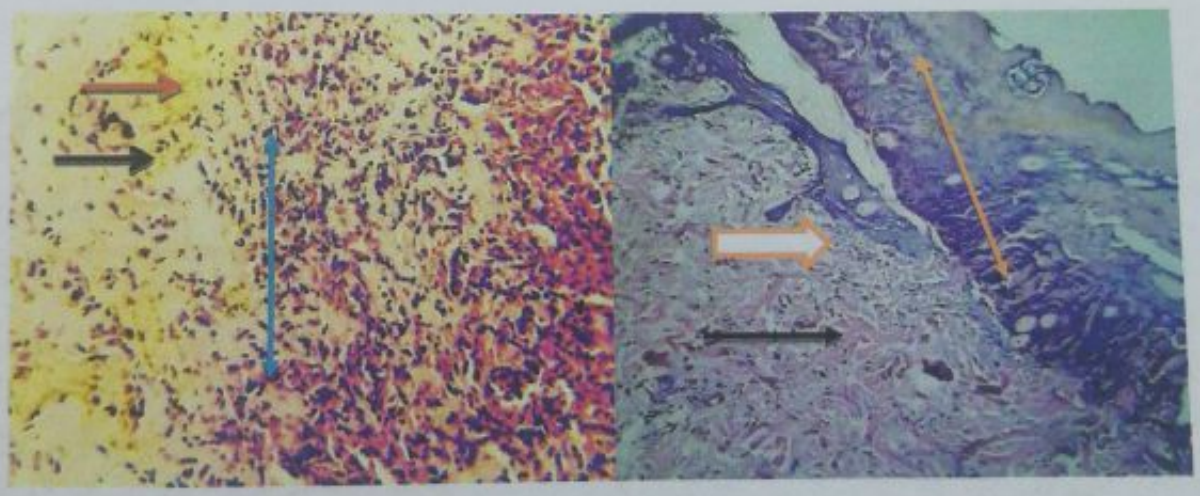

Fig-5. Histolpathological section in skin incision of group (T)on day seven post-incision shows neutrophils,mononuclear cells and fibroblasts cells (blue arrow) with hemorrhage (red arrow) in necrotic foreign materials over the incision (black arrow) (H&E stain; 10X).

Fig-6. Histolpathological section in skinincision of group (A) on day seven post-incision shows initiated extentionof several layers of epithelial cells(thick arrow)under neutrophils,macrophages,infiltration of the foreign materials with fibroblasts proliferation (long arrow) and over granulation tissue(black arrow) (H&E stain; 10X).

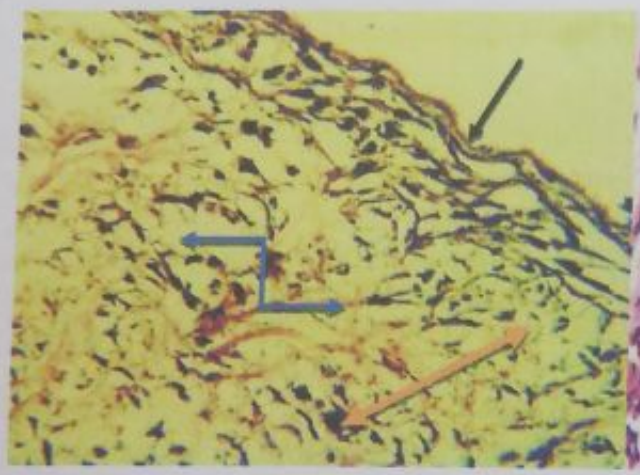

Fig-7. Histolpathological section in skin of group (T)on day 14 post-incision shows thin epithelial cells (black arrow)over granulation tissues consisting from congested blood vessels with irregular collagen fibers (blue arrow) and inflammatory cells infiltration (orang arrow)(H&E stain; 10X).

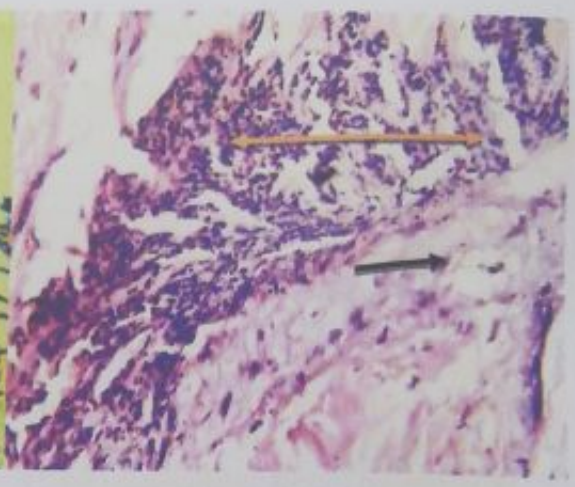

Fig-8. Histolpathological section in skin of group (A) on day 14 post-incision shows several layers thickness epithelial cells (lower arrow) extended under inflammatory and cellular debris (upper arrow) (H&E stain; 40X).

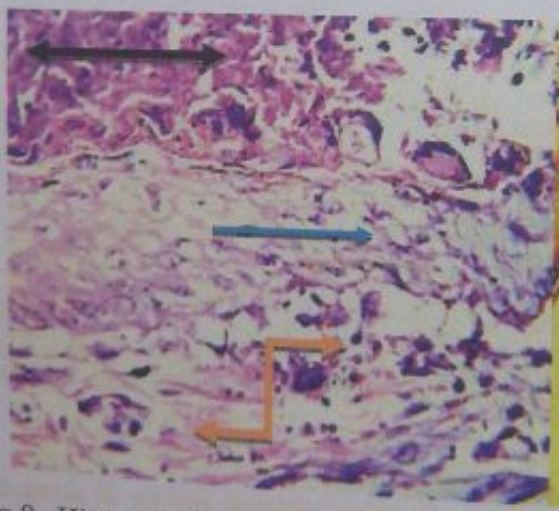

Fig-9. Histopathological section in skin of group (T)on day 21 post-incision shows thickness layers of epithelial cells (middle arrow)extended under inflammatory and cellular debris (upper arrow) and over mature granulation tissues (lower arrow) (H&E stain: 10X).

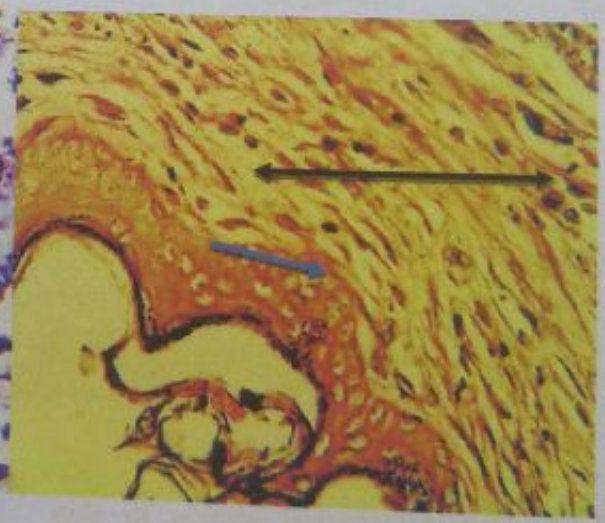

Fig.10-Histopathological section in skin of group (A)on day 21 post-incision shows epidermal layer (lower arrow)over mature granulation tissues (upper arrow)(H&E stain; 40X).

DISCUSSION

In the present study signs of mild redness and swellingmay be attributed to inflammation resulted from wounding and these signs are common complications following most surgical operations. With prefect post-operative cares these signs diminished rapidly and the animals retained to its normal condition and activity. This interpretation come in line with (11). Healing times and hair growth of the autograft transplants were lesser than that recorded in allogeneic graft, these result was agreed with (12 and 13) whom recorded wound healing in (18 - 20) and (21 - 22) days respectively.

Forty-five examined grafts showed favorable healing outcomes and the wounds appeared similar to normal skin especially in autograft group. Skin grafts fail in one rabbit related to autograft group, this may be due to fluid accumulation beneath the graft (seroma) Similar findings were noticed by (14) in rabbits. The failure of two grafts in allogeneic group may be ascribed to hematoma (due to improper arrest of bleeding from surgical incision) in the first rabbit and infection in the second one. A study by(15) indicate that infection, inflammation or fluid accumulation beneath the graft were the most common causes of failure. Infection can be avoided by carefully preparing the wound bed and applying wet saline dressings that are changed every 4 hours. In another research (16) referred that blood, serum or exudate accumulated beneath a graft prevents fibrin from attaching the graft to the wound in addition to impairment of vascularization from the wound into the graft. A study by (17), indicated that hematomas and seromas act as a block to link-up of the outgrowingcapillaries,thus it must be drained to facilitate graft survival.

The histopathological pictures of the present study were similar to that mentioned by (18 and 19). The presentresults showed good prognosis (healing) in auto-grafts group, which reflected an inflammatory reaction characterized by present of neutrophils and mononuclear cells infiltration (macrophages).

Neutrophils are the first cells arrive to the wounded area and constitute the first line of defense against bacterial infection. Neutrophils release proteolytic enzymes that help in wound debridement (20). Then macrophages which appear in day 5 after surgery and gradually replaced neutrophils in 7 and 14 day post- surgery, can play animportant role in wound healingas they involved in the inflammatory process, in lymphocyte activities, in tissue debridement and bactericidal activities as well as in tissue remodeling (21) Macrophages secrete a variety of important growth factors which

perform several important functions; chemo-attraction of several cellular components, stimulation of angiogenesis, stimulation of granulation tissue and extracellular matrix formation (22).

Mature granulation tissue was noticed in the third week post-wounding. A study by (23) referred that granulation tissue is highly cellular and vascular tissue in the wound that acts to clear all the debris from the wound, provides a framework to fill the tissue defect and supports the regenerating epithelium, and helps to nourish the newly forming mesenchymal tissue and also may involve in nourishing the newly transformed skin

In conclusion the autograft achieved the best result because the healed incision was similar to normal skin, thus it is more suitable for use as a skin substitute in comparing with allograft type.

دراسة مقارنة بين الرقع الذاتية والرقع من نفس النوع لاصلاح الجروح الجلدية في الارانب

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الخلاصة

هدفت الدراسة الحالية لاستعمال نوعين من الرقع لاصلاح الجروح الجلدية في الارانب بالاعتماد على المعايير السريرية والنسجية-المرضية. استعمل للدراسة (24) ارنبا بالغا قسمت عشوائيا الى مجموعتين: مجموعة الرقع الذاتية و مجموعة الرقع من نفس النوع وبواقع (12ارنب\مجموعة). استعملت كبريتات الاتروبين قبل التخدير العام الذي تمثل بمزيج الكيتامين-الزيلزين.رقدت الارانب وتم عمل جرحين سعة (4×4) سم امامي وخلفي على ظهر كل ارنب (عدد الجروح الكلي هو 48 جرحا) ثم عمل فصل أعمى للجلد بغية فصله وازالته. ثبتت الرقع في كلا المجموعتين بغرز بسيط متقطع وبخيط الحرير بعدها غطيت الجروح بشاش معقم غير لاصق تم تبديله كل 24- 72 ساعة وحقنت الحيوانات المضاد الحيوي. أخذت عينات للفحص النسجي-المرضي بعد (7و14و21) يوما من اجراء الجروح.

أظهرت النتائج السريرية حدوث التهاب بسيط (احمرار و تورم) بعد اكمال خياطة الجروح. كان وقت الشفاء في مجموعة الرقع الذاتية هو(15 - 17) مع فشل رقعة في احد الارانب. اما وقت الشفاء في مجموعة الرقع من نفس النوع هو 21) (19 -مع فشل رقعتين في ارنبين .

بين الفحص النسجي- المرضي في كلا المجموعتين وجود خلايا التهابية في الاسبوع الاول من احداث الجروح ومع تقدم الوقت تكون النسيج الحبيبي. وكانت سرعة شفاء الجروح في مجموعة الرقع الذاتية اسرع عند المقارنة مع مجموعة الرقع من نفس النوع.

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