Effect of application of Chitosan in skin grafting surgeries in rabbits

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Abstract. A study was done at the theatre of department of veterinary Surgery & obstetrics, College of Veterinary Medicine, University of Kerbala, Karbala, Iraq which performed to compare the effect of application of chitosan action as scaffold with collagen fibers. The histopathological findings in operation's sites showed the developments of skin graft's healing which was the guide to monitoring the healing. The results showed that the chitosan-treated grafts was healed more controllable with less scar tissue than non-treated grafts which indicates that the chitosan was coordinated the healing.

Keywords. Chitosan, Skin grafting, rabbits.

1. Introduction

Chitosan is derived from chitin which is the second maximum copious biopolymer after cellulose so it is biodegradable polymer and it accelerates wound healing [1]. It has been described that chitosan authorized regeneration of tissue elements in skin wounds and has affected positively in healing of wounds. Also it appear many benefits for topical applications, including non-irritancy and some antibacterial actions [2]. This study was aimed to study the availability of the applications of chitosan in healing of skin. Skin grafting a technique mainly used or reconstruction of skin defects at the last decades. The techniques of flaps and grafts considered are the two common surgeries utilized for restoration of losing of the tissue. Simple skin graft used as a thin layer harvested with a special scalpel called dermatome [3]. A free skin graft is a piece of skin that completely separated from normal area and transferred to a wound to a wound at another site that establish new vascular connections to survive. Skin grafting consider the best consideration for the wide wound closure or deep burns of wide body areas [4]. Skin grafting had been contraindicated if inosculation from receiver bed is unconfident or unpredictable or n wound infection [5]. Chitosan was discovered in 1859 by Rouget . Since then, it has been used as a pharmaceutical excipient and to promote wound healing [6]. Chitosan has been able to bind to substances, including acids, lipophilic materials and minerals [7] as well as inhibit fat absorption. The origin of chitosan is a fiber product that is obtained from deacetylated chitin. Chitin is naturally occurring substance found in the shells of crustaceans, invertebrates and fungi [6]. Chemical composition of chitosan consider as a copolymer which comprises of β – (1→4) – linked 2- acetamino- 2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-
glucopyranos units that obtained by alkaline deacetylation of chitin which is the main component of the exoskeleton of crustaceans [8]. Chitosan has a structure too similar to cellulose; which is fairly reactive compounded its formed in many forms such as powder, paste, film and fiber. The solubility of chitosan in dilute aqueous acetic, lactic, succinic and formic acids [9] (Shakeel et al., 2014) . Commercial production of chitosan by deacetylation of chitin [10]. Wound dressing, trauma, burns, dermatitis, purulent diseases, septic pod dermatitis, interdigitalphlegmon, interdigital hyperplasia, canker [11, 12, 13]. Wound infections are manifestations of disturbed host bacteria equilibrium in a traumatized tissues condition in favor of the bacterium, the wound infection not only have the possibility to emerge a systemic restraint (sepsis) but it was highly probable to prevent the multiple processes involved in the orchestrated progression of normal wound healing, each process involved in healing are affected when bacterium proliferated in the wound [2]. Chitosan considered as non-toxic antibacterial polymer have the ability to prohibit a wide variety of fungi, bacteria, viruses and yeasts which its interaction had been experimented in vitro and in vivo [14]. The accurate mechanism of the antimicrobial actions of chitosan are still hesitating, it has been suggested that is interactions between negatively charged microbial cell membrane and positively charged chitosan molecules can lead to the disturbance of microbial membrane, and thereafter infiltrate intracellular ingredients [15, 16]. Chitosan has exert as anti-inflammatory effects by restrain cyclooxygenase-2 protein expression and prostaglandin E2 and decline pro-inflammatory cytokines (e.g. interleukine-1β tumor necrosis-α). Otherwise, chitosan treatment raises the expression of the interleukin-10 [17]. Skin grafting is classified depending on the relation between the donor site and the recipient bed as: Autograft or autologus, which the skin grafted from different site on same body (man or animal). Isograft, syngraft or isogenic, which the grafting performed between animals or men are genetically identical (e.g. monozygotic twins). Allograft or allogenic, which the grafting performed between animals with same species, or men but genetically non-identical. Xenograft, heterograft or xenogenic, which the grafting performed between different species.[18].

2. Materials and Methods

2.1. Experimental design

Eighteen adult female rabbits divided randomly to two groups; the first group (A) which compel to skin grafting by transplantation from forelimb to hind limb using chitosan as pad under the graft and the second group (B) which compel to same grafting in (group A) without addition of chitosan application on grafting area.

2.2. Chitosan

The chitosan which used in this experiment is manufactured Vitex Pharmaceuticals ®; Australia. The experiment done at the theatre of department of veterinary surgery and obstetrics, college of veterinary medicine / University of Kerbala, Karbala, IRAQ. Study parameters were hematological parameters and histopathological findings 21 days post graftings and compare the healing of the skin according to gross findings and histopathology.

2.3. Surgical operation

2.3.1. Preoperative preparation

The animals of this study were prepared to surgery by fasting for 12 hours withheld of food and 6 hours of water before the operation, the area of medial tibial region was clipped and shaved and prepared aseptically. The skin was scrubbed by bovidine – iodine 2.5%. The rabbits were put at a lateral recumbency and covered with surgical drapes which fixed to the skin with towel clips.
2.3.2. Anesthesia

All the operations were performed under general anesthesia by intramuscular administration the combination of Xylazine and Ketamine at the dose (3:35 mg per kg B.W.) with additional dose of Xylazine to increase depth and prolong anesthesia [19, 20, 21].

2.4. Surgical technique

2.4.1. Group A

The technique which performed in this experiment was the transplantation of a square – shape of skin with 1cm×1cm in diameter from forelimb transplanted to a gap made in hind limb filled with chitosan powder then the borders of graft sutured with original skin by simple interrupted suturing covered by gauze pad changed every two days with 5 postoperative days of treatment with penicillin-streptomycin mixture to prevent any type of infection (Figure 1).

![Figure 1](image-url)

*Figure 1.* Shows the stages of experiment's operation: (A) Ablation of the graft from intact skin. (B) Preparation of grafting site. (C) Implanting of graft with application of chitosan.

2.4.2. Group B

The technique had been done same as in group A without adding of Chitosan at the site of operation. Other provisions was done consequently.

2.5. Postoperative care
The treated animals were housed in semi-opened house as confine within house garden fed with grain and vegetables along the period of experiment receiving freely water and food (containing green grass and grain). Experimented animals were administrated with mixture of Penicillin and streptomycin with a dose of (20000 I.U : 10 mg per kg B.W.) for three days post operatively. Diclofenac sodium in a dose of 1mg/kg B.W. were administered intramuscularly for 3 days post operatively. Rectal temperature, respiratory rate and heart rate were recorded daily for three days post operation.

3. Results and Discussion

The study showed that usage of chitosan as application with skin grafting was possible and useful. A total of 18 rabbits underwent successful healing with or without chitosan application, and it was observed that the normal clinical progress was without any clinical sign of complication or death due to the skin grafting and animals were in good general health and clinical condition, unless a mild signs of inflammatory signs in chitosan treating animals at first 2 days. The animals continued food intake directly after surgery with normal intensification of body weight. Understanding of the clinically and patience were very important for success of technique. However our previous experience showed that the chitosan-treated animals showed, in post mortem finding, most successful skin healing compare with non-treated animals, in addition to that there were reduce time of operation site's injury healing in chitosan-treated animals, reduced recovery time and postoperative wound complications.

3.1. Postoperative observation

The surgical phase of anesthesia was attained by administering Xylazine + Ketamine in all the animals. Injectable anesthesia was the method of choice for maintaining anesthesia during the procedure. It was perfectly done in rabbits in this experiment, and the animals completely lost consciousness without response to pinprick with excellent muscle relaxation. The post-surgical recovery from anesthesia in the two experimental groups were uneventful. The intramuscular administration of Xylazine with a dose of 3mg/kg B.W. and Ketamine with a dose of 35 mg/kg B.W. were given one by one injection produced about 45 minutes duration of general anesthesia with additional Xylazine could be added if depth of anesthesia was not adequate without any significant complication that might occur as regurgitation, delayed recovery. The surgery of indication of skin grafting in both groups were performed successfully in all experimental animals. During the transplantation of skin graft; no trauma or injury to any muscle, blood vessels or nerve that could happen. At the beginning of the study consumed longer duration time for the chitosan- treatment's technique, but with more experience, the time became shortened, the maximum time required to obtain the chitosan application skin grafting 35- 45 while the duration time of non-treated animals 30-40 minutes.

3.2. Post-operative findings

3.2.1. Zero time

The Post-operative findings showed normal traumatic reactions with clear edges of wounds at the site of operation in both of groups immediately after the operation (Figure. 2).
Figure 2. Fig 2: shows the site of operation in both groups (time zero)

Twenty first days Post-operatively; the site of operation appear the complete healing with appearance of hair growth in grafting area. Hematological parameters shows no significant differences between both groups or within each group.

3.3. Histopathological findings

Group (A): a good healing signs with many hear follicles which grows within grafting tissue, with a thin line of scar tissue, Although, an accumulation of few inflammatory cells (Figure 3).

Group (B); the healing signs shows the hear follicles which grows within grafting tissue but less the which appeared in group A, with marked line of scar tissue, However, a negligible accumulation of inflammatory cells (Figure 4).

Figure 3. Shows the healing in group (A) which appear the growth of hair follicles (A) revascularization (B) with perfect scar formation(C), (X10). (C is not visible in both picture, make it like A and B). (C ) refer to the area of scar tissue area which histologically classified as granulation and fibrous tissue (appear as white area).
4. Discussion

In this study, it was appeared that Chitosan play a significant role in skin grafting healing similar with [22] that chitosan accelerate wound healing, as well as controlled the healing because the chitosan shared with other materials to scaffold the wound gap. this findings agreed with [23] and same with [24] who appeared its effect on bone marrow stromal cell osteogenesis and angiogenic factor secretion. Totally the organization of skin grafting healing which showed in chitosan-treated group in rabbits is followed the conclusion of [1] that chitosan, the biodegradable polymer, is enhance wound healing. In conclusion, the application of chitosan as filling the gap under graft can be done successfully in rabbits, despite the fact that Chitosan act as raw fibers in collagen-coated under the skin. Although, the chitosan accelerate healing of the skin grafting as well as it coordinate and controlled the scar formation.

5. References

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