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PREPARATION AND EVALUATION OF CROSS LINKED CHITOSAN-POLYURETHANE MESH IN TISSUE REPAIR IN SHEEP

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ABSTRACT

In the present study, an interpenetrating polymer network (cross-linked chitosan-polyurethane mix) was successfully prepared. It was found that the mixing of 70% chitosan and 30% polyurethane gave the best results as far as the mechanical properties of the network are concerned. Toxicological evaluation of the network was done in 15 rabbits, and it was found to be pathologically non-toxic. The possible effects of the network on the liver, kidneys, and skeletal muscles of sheep with clean or infected open wounds were studied through the measurement of plasma aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and acid phosphatase. In sheep with clean open wounds that were implanted with the network, the levels of the enzymes were 11.04 ± 0.54; 30.83 ± 1.20; 36.37 ± 1.52; and 5.52 ± 0.37, respectively. In sheep with contaminated wounds, the levels of the enzymes were 11.29 ± 0.30; 38.58 ± 1.16; 38.20 ± 1.21, and 6.31 ± 0.46, respectively. Statistically significant differences were not encountered in the values of alanine aminotransferase, alkaline phosphatase, and acid phosphatase in sheep with clean wounds and those with contaminated wounds. However, the level of aspartate aminotransferase was higher significantly ($P<0.01$) in sheep with contaminated wounds than in sheep with clean wounds. Histologically, the healing of the clean and contaminated open wounds proceeded through the same processes as described in the literature. Bacterial infection was not observed in any of the wounds that were implanted with the network.

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تحضير وتقييم مشبك من الكايتوسان المتشابك عرضياً والبولي يوريثين واستخدامه في اصلاح الانسجة اللينة في الضأن

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الخلاصة

في هذه الدراسة تم وبنجاح تحضير شبكة بوليمرية متداخلة (مزيج من الكايتوسان المتشابك عرضياً مع البولي يورثين). ووجد بأن خلط الكايتوسان بنسبة ٧٠% والبولي يورثين بنسبة ٣٠% قد اعطى

افضل النتائج من ناحية قوام الشبكة ومواصفاتها الميكانيكية. وتم اجراء التقييم السمي للشبكة بغرسها في (١٥) أرنباً ودراسة الكبد والكلى والامعاء نسجياً بعد فترات ٢٤ و ٤٨ و ٧٢ ساعة بعد الغرس حيث وجد بأن الشبكة غير سامة. وتمت دراسة التأثيرات المحتملة للشبكة على الكبد والكلى والعضلات الهيكلية في الاغنام التي احدثت فيها جروح جلدية مفتوحة نظيفة (المجموعة الاولى) وجروح جلدية مفتوحة ملوثة (المجموعة الثانية) من خلال قياس خمائر aspartate aminotransferase و alanine aminotransferase و الفسفتاز القلوية والفسفتاز الحامضية في بلازما الحيوانات. وفي الضأن الذي احدثت فيه جروح مفتوحة نظيفة كانت قيم الخمائر ١١.٠٤ ± ٠.٥٤، ٣٠.٨٣ ± ١.٢٠، ٣٦.٣٧ ± ١.٥٢، ٥.٥٢ ± ٠.٣٧، على التوالي. أما في الضأن الذي احدثت فيه جروح مفتوحة ملوثة فقد كانت قيم الخمائر ١١.٢٩ ± ٠.٣٠ ، ٣٨.٥٨ ± ١.١٦، ٣٨.٢٠ ± ١.٢١، ٦.٣١ ± ٠.٤٦، على التوالي. ولم تلاحظ فروق معنوية احصائياً بين قيم خمائر alanine aminotransferase والفسفتاز القلوية والفسفتاز الحامضية في مجموعتي الضأن. ومع ذلك فقد كان مستوى خميرة aspartate aminotransferase اعلى عند مستوى احتمالية ($P<0.01$) في الضأن الذي احدثت فيه جروح مفتوحة ملوثة (المجموعة الثانية) من الضأن الذي احدثت فيه جروح مفتوحة نظيفة. ونسيجياً لوحظ بأن التئام الجروح النظيفة والملوثة قد جرى من خلال آليات مماثلة لتلك الموصوفة في النشريات العلمية. ولم يحدث خمج جرثومي لأي من الجروح (النظيفة والملوثة) التي تم غرس الشبكة فيها.

INTRODUCTION

Chitin is a linear homopolymer of β (1, 4)-linked N-acetyl-D-glucosamine, and chitosan is a partially deacetylated chitin (1-4). Chitin is widely distributed in nature as the skeletal material of crustaceans and insects, as well as a component of cell walls of bacteria and fungi, and is the second most abundant polymer occurring in nature, after cellulose. Chitosan lacks irritant or allergic effects and is biocompatible with both healthy and infected human skin (1). When chitosan was administered orally in mice, the LD_{50} was found to be in-excess of 16 g/kg, which is higher than that of sucrose (1). The intriguing properties of chitosan have been known for many years and the polymer has been used in the fields of agriculture, industry, and medicine (5-7). In agriculture, chitosan has been described as a plant

antivirus, an additive in liquid multicomponent fertilizers and it has also been investigated as a metal-recovering agent in agriculture and industry (1). Chitosan has been noted for its application as a film-forming agent in cosmetics, a dye-binder for textiles, a strengthening additive in paper and a hypolipidic material in diets (1, 8). It has been used extensively as a biomaterial, owing to its immunostimulatory activities, anticoagulant properties, antibacterial and antifungal action and for its action as a promoter of wound healing in the field of surgery (9-19). In addition, chitosan has a variety of promising pharmaceutical uses and is presently considered as a novel carrier material in drug delivery systems, as indicated by the large number of studies published over the last few years (20-24). The purpose of this study were to prepare a new interpeneterating polymers networks (chitosan-polyurethane meshs) (IPN) and to evaluate the application of these networks in the treatment of experimentally- induced skin injuries in sheep.

MATERIALS AND METHODS

1. Preparation of chitosan:

To isolate and purify the chitin, the exoskeleton of shrimps were isolated, thoroughly cleansed in water, dried in an electrical oven at 100°C, and grinded till a smooth powder was obtained. A hundred grams of the powder was mixed with one liter of 5% HCL and the mixture was agitated continuously for 24 hrs at room temperature (demineralization). The solution was filtered through a clean piece of clothes and the precipitate was cleansed with water for several times to get rid of residues of the acid. A one liter of 50% sodium hydroxide (NaOH) was added to the precipitate and the mixture was agitated continuously at 90°C and for 3 hrs to get rid of the protein (deproteinization). The mixture was then left to cool, filtered, rinsed with distilled water for several times to obtain chitin in a ratio of 35% of the quantity used (25-26). Chitosan was obtained by adding 250 ml of potassium hydroxide (50%) to 20 gm of the isolated chitin. The mixture was then heated at 90°C for 6 hrs with continuous agitation. This process was repeated for 3 times, and the precipitate was rinsed and dried. The chitosan was obtained at 75% of the quantity used (25-26).

2. Chitosan crosslinking:

Cross linking of chitosan was done through dissolving the chitosan in 0.1 M HCL. The cross linking material (glutaraldehyde) was then added to the mixture at the rate of 8 mg/ml. Neutralization of the mixture was done through the addition of 0.1M sodium hydroxide till a swollen precipitate was obtained which represented the cross linked chitosan (26).

3. Preparation of the IPN:

The IPN were prepared through the mixing of the cross linked chitosan with polyurethane. The cross linked chitosan was mixed with methylene diphenyl dissocyanate so that the later enter the spaces of the cross-linked chitosan. Polyester polyol was then added to the mixture in order to allow the reaction between the methylene diphenyl dissocyanate and the polyester polyol. The formation of foam and the emission of Co_2 are indications for the occurrence of the reaction. In order to obtain the best results concerning flexibility and homogeno of the prepared IPN, the proportions of chitosan and polyurethane were

changed as shown in Table (1). The 70% of chitosan was found to be the most suitable and was used in the experiments.

Table 1. Percentages of mixing of chitosan and polyurethane.

IPN'S No.	Chitosan (%)	Polyurethane (%)*
1	10	90
2	20	80
3	30	70
4	40	60
5	50	50
6	60	40
7	70	30
8	80	20
9	90	10

* Equal percentages of methyl diphenyl dissocyanate and polyester polyol.

4. Physical and chemical evaluation of IPN:

Diagnosis of chitin and chitosan and the IPN were done by the infrared technique (FTIR 84005, Shimadzu Co.) [*]. The technique includes mixing a quantity of the specimen with potassium bromide (KBr). Following good mixing, the mixture was encysted under the pressure of 15 tons, to obtain a thin disc, which later subjected to examination and measurements.

The tensile strength of the IPN was measured by an Instron apparatus type 1193[*]. The specimen which is made into dumbbell shape is placed between the two levers of the apparatus and pulled at a speed of 5mm/second till the specimen is cut, and the tensile strength was read at this point.

5. Toxicological evaluation of the IPN:

Fifteen adult male rabbits of a local breed and weighing 1.5-2 kg were used in this experiment. Following acclimatization, the rabbits were allocated into 3 equal groups, and the gluteal region of each rabbit was prepared routinely for surgery. Each rabbit was premeditated with 10mg/kg acepromazine maleate[**] intramuscularly to induce tranquilization. After 10 minutes, the animal was given intramuscularly a mixture of ketamine hydrochloride[***] (35 mg/kg) and xylazine[****] (5 mg/kg), (27). The IPN was disinfected with ultraviolet radiation at a wave length of 250-280 nm and for 15 minutes. A full thickness skin piece (3×4cm) was removed, and the IPN was placed in the defect and fixed from the margins and center of the wound using a 3 0 absorbable suture material (28-29). The animals were then euthanatized at the rate of 5 rabbits at each of the 24, 48, and 72 hrs post-surgery. Tissue specimens were collected from the liver, kidneys, and intestines, and fixed in 10% formalin for 48 hrs, trimmed to suitable sizes,

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[**] Calmivet, Vet Oquinol, S. A. Veterinary, MAGNY-Vernois-70200.

[***] Ketallar, 50 mg/ml, Park Davis and Company, Ponty pool, Gwent, U.K.

[****] Rompun, Pantex Holland, B.V., De. Hoeve 28, 20 mg/ml.

washed, dehydrated, cleared in xylol, embedded in paraffin wax, sectioned at 5-6 μm thickness, stained with hematoxylin and eosin, and examined with a light microscope (30).

6. Application of the network for treatment of clean cutaneous wounds in sheep:

Twenty four adult Awasi sheep of both sexes were used in this study. The gluteal region of each rabbit was prepared for aseptic surgery. Each animal was given a mixture of ketamine HCL (4 mg/kg) and xylazine (0.05 mg/kg), as well as diazepam (2 mg/kg) intramuscularly. Following induction of anesthesia, a full thickness skin piece (5× 6 cm) was excised and replaced by the IPN which was fixed in situ by an 3 0 absorbable suture material. Biopsy specimens were collected from the wounds at 7, 15, 30, 60, 90 and 120 days post operation. The tissue specimens were fixed in 10% formalin for 48 hrs, processed, and examined as mentioned previously.

Blood samples were collected from all sheep and preserved in plastic test tubes devoid of anticoagulant and left in oblique position at room temperature. The blood serum was separated by centrifuging the tubes at 3000 rpm for 15 minutes, and the serum was preserved at –20°C. till analysis and measurements of the enzymes which included AST (aspartate aminotransferase), ALT (alanine aminotransferase), alkaline phosphatase (ALP), and acid phosphatase (ACP). A Biomaghreb test kit was used to measure the enzymes following the Cecil colorimetric method (French made apparatus) at a wavelength of 490-520 nm (Clinical Pathology Unit, the Gurna General Hospital).

7. Application of the network for treatment of contaminated wounds in sheep:

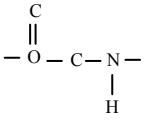
Twenty four adult Awasi sheep of both sexes were used in this study. Full thickness skin piece (5× 6 cm) was excised aseptically from the gluteal region of each rabbit and the wounds were left open for 24 hrs. The defect was then replaced by the IPN as described in the previous group. Collection of biopsies, fixation, processing, and examination were done as described in the previous group. Similarly, AST, AIT, ALT, and ACP were determined as described in the first group.

8. Statistical Analysis:

Results of mechanical properties and measurements of enzymes were analyzed statistically using a one-way analysis of variance. When a statistically significant difference ($P < 0.05$) was obtained, Tukey-HSD test was then performed (31).

RESULTS

Results of infrared analysis of the chitosan and the network prepared from it are shown in figures 1 sand 2, respectively. From data presented in these figures, the sites of frequencies of the functional groups were ascertained and are presented in table (2). The frequency of absorption of the NH_2 group appeared as two bands in the range of 3300-3400 cm^{-1} (Fig.1). In figure 2, the NH_2 group disappeared and replaced by OH group at the site 3400 cm^{-1}. Similar absorption bands of CH2, C-O-C, and C–C groups are evident in figures 1 and 2. The frequency of absorption of urethane group occurred at the sites of 1300 and



1700 cm^{-1}, (Fig.2). This frequency is not seen in figure 1 and this means the occurrence of a reaction between the chitosan and the polyurethane.

Table (3), presents results of mechanical evaluation of the IPN (Cross-linked chitosan-polyurethane) as compared to cross-linked chitosan alone. From these data it became obvious that the IPN is more flexible and withstand more stress than the cross-linked chitosan alone. Furthermore, the possible force inflicted on the surface area of the cross section of the polymeric network depends on strength, flexibility, strain, and elasticity as shown in figure 3 which illustrates the types of stress-strain curve for some elastic polymers.

Gross and microscopic pathological lesions were not encountered in the viscera of rabbit in which the IPN were implanted for 24, 48, and 72 hrs.

Table (4), presents the serum values of ALT, AST, ALP, and ACP in animals with clean wounds and animals with contaminated wounds. Statistical analysis of these values have indicated that the levels of SGOT, ALP, and ACP in the first group of sheep were 11.04 ± 0.54, 30.83 ± 1.20, 36.37 ± 1.52, and 5.52 ± 0.37, respectively. In the second group of sheep, these values were 11.29 ± 0.30, 38.58 ± 1.16, 38.20 ± 1.21, and 6.31 ± 0.46, respectively. Statistically significant differences were not encountered between the ALT, ALP, and ACP values of sheep with clean wounds and those with contaminated wounds. However, the AST level was higher significantly ($P<0.01$) in sheep of group 2 (with contaminated wounds) than in sheep of the first group (with clean wounds).

Figure 1: Infrared analysis of the cross-linked chitosan. The frequency of absorption of NH_2 group appeared as two bands in the range of 3300-3400 cm^{-1}.

Figure 2: Infrared analysis of the IPN. Note that the NH_2 group disappeared and replaced by OH group at the site 3400 cm^{-1}.

Table 2. Sites of frequencies and bonds and the functional groups of the chitosan and the IPN.

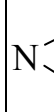
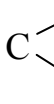
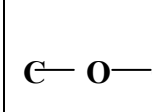
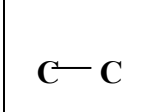
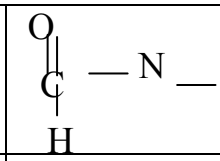
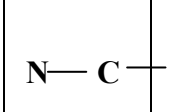
Compound / Group		**OH**					
Cross-linked Chitosan	3300-3400	3400	2940	1068	1460-1590	----	----
IPN	---	3400	2940	1068	1460-1590	1300	1700

Table 3. Results of examination of the tensile strength of the IPN and the chitosan alone.

Specimen	T_b kg /cm^2	E %
Cross – linked chitosan	21.8	0.96
IPN	28.9	1.2

T_b represents the tensile strength at the point of rupture, and E represents the elasticity.

Figure 3: The types of stress-strain curves for some elastic polymers.

Table4: The levels of enzymes in sheep with clean wounds (group 1) and sheep with contaminated wounds (group 2).*

Enzyme	Group	No. of Animals	Mean	Standard Deviation	Standard error
SGPT	1	24	11.04	2.67	0.45
µ/dl	2	24	11.29	1.51	0.30
SGOT	1	24	30.83	5.88	1.20
µ\dl	2	24	38.58	5.70	1.16
AP	1	24	36.37	7.46	1.52
I.U./L	2	24	38.20	5.94	1.21
ACP	1	24	5.52	1.84	0.37
I.U./L	2	24	6.31	2.28	0.46

*** SGOT** was higher significantly ($P<0.01$) in the second group than in the first group. No significant differences were encountered in the rest of the parameters.

Histological examination of the clean wounds that have been induced in sheep of group (1) revealed increased thickness of the epidermis close to the wound line. The wound defect was filled with a dense mature granulation tissue that consisted of large numbers of fibroblasts and bundles of collagen fibers (Fig. 4). Although the fibroblasts were haphazardly arranged in the wound defect, the majority of them were horizontally arranged in relation to the wound line. Similarly, the collagen fibers were arranged as bundles horizontally oriented relative to the wound line. Few newly-formed blood vessels were seen in the granulation tissue. Large numbers of IPN crystals were also seen in the

granulation tissue (Fig. 4). These crystals were surrounded by numerous mononuclear cells (lymphocytes, plasma cells, and macrophages) and foreign-body giant cells (Fig. 5). At the 15th postoperative day, the histology of the wounds was similar to that seen in the 7 days old wounds. Additionally, a large number of mononuclear cells and foreign-body giant cells were seen around the IPN crystals (Fig. 6). At this period, many of the IPN crystals disappeared from the wound tissue. At the 30th, 60th, and 90th postoperative days, a gradual decrease of the IPN crystals was seen (Fig. 7). At 120 days following infliction of the wounds, it was difficult to recognize the wound line and the IPN crystals disappeared completely.

In contaminated wounds at the 7th postoperative day, the wound defect was filled with mature granulation tissue that was arranged horizontally relative to the wound line (Fig. 8). Few IPN crystals surrounded by large numbers of erythrocytes were seen in the granulation tissue. At the 15th postoperative day, large numbers of mononuclear cells and foreign-body giant cells appeared in the granulation tissue particularly around the IPN crystals (Fig. 9). Many of these crystals were degraded into small fragments. At the 30th postoperative day, the wound defect was filled with mature granulation tissue that contained mononuclear and foreign-body giant cells arranged around IPN crystals (Fig. 10). The IPN crystals were not seen at the 60th postoperative day. At the 90th postoperative day, the site of the wound has healed completely and the tissue regained its normal shape and structure. At 120 days following infliction of the wounds, the site of the wound was normal histologically.

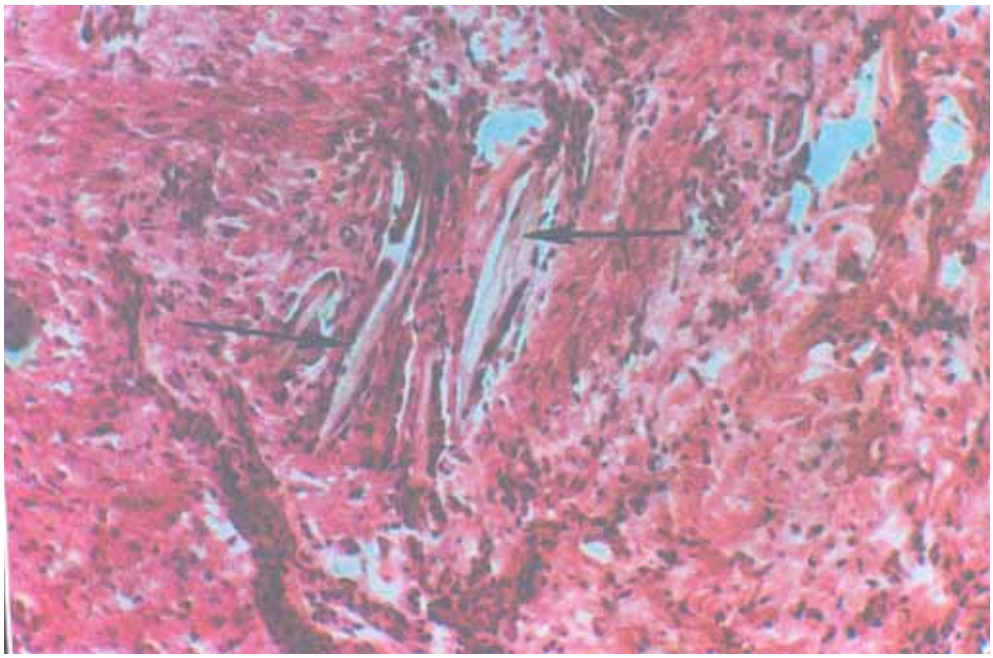


Figure 4: Cross section of a clean open skin wound in sheep at the 7th postoperative day. The wound defect is filled with a mature granulation tissue. Note the presence of fragments of the IPN in the granulation tissue (arrows). H & E. 40X.

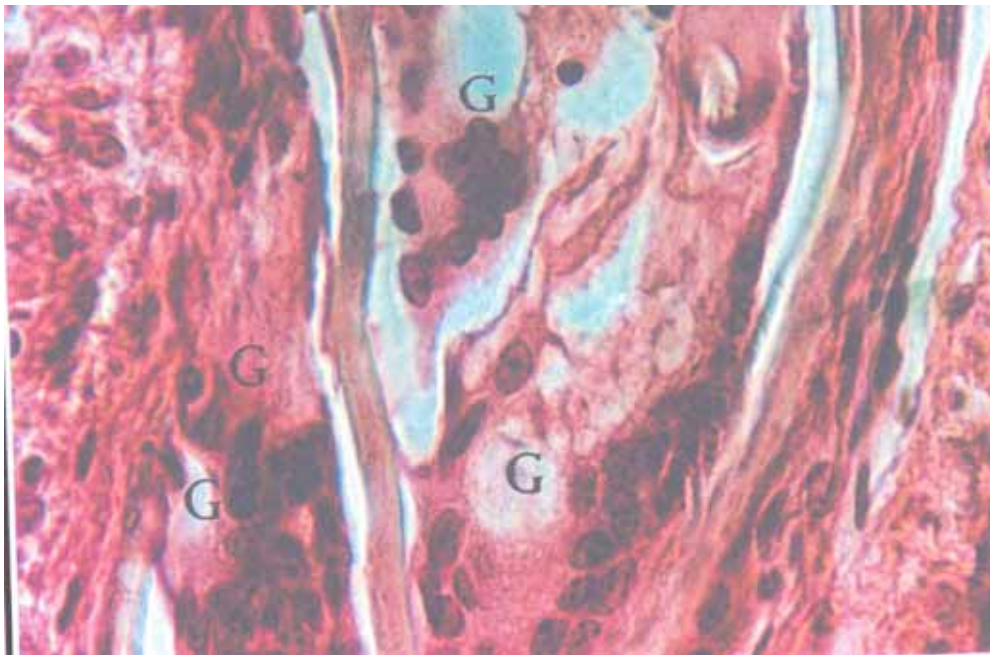


Figure 5: Cross section of a clean open skin wound in sheep at the 7th postoperative day. Crystals of the IPN are surrounded by foreign-body giant cells (G). H & E. 160X.

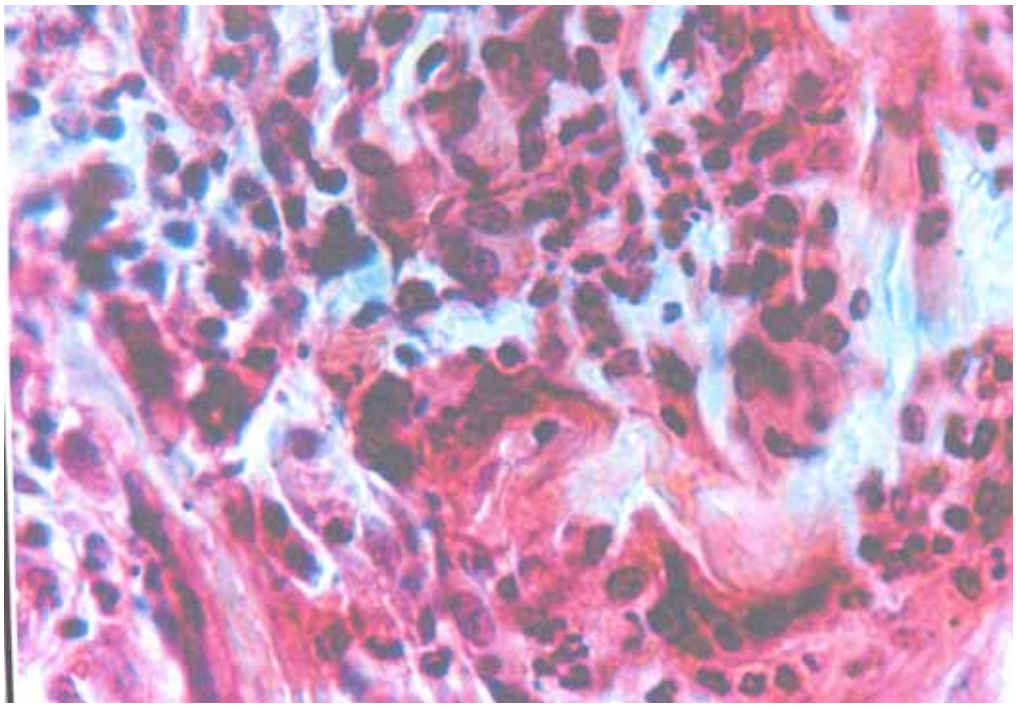


Figure 6: Cross section of a clean open skin wound in sheep at the 15th postoperative day. Mononuclear and foreign-body giant cells could be seen around remnants of the IPN. H & E. 160X.

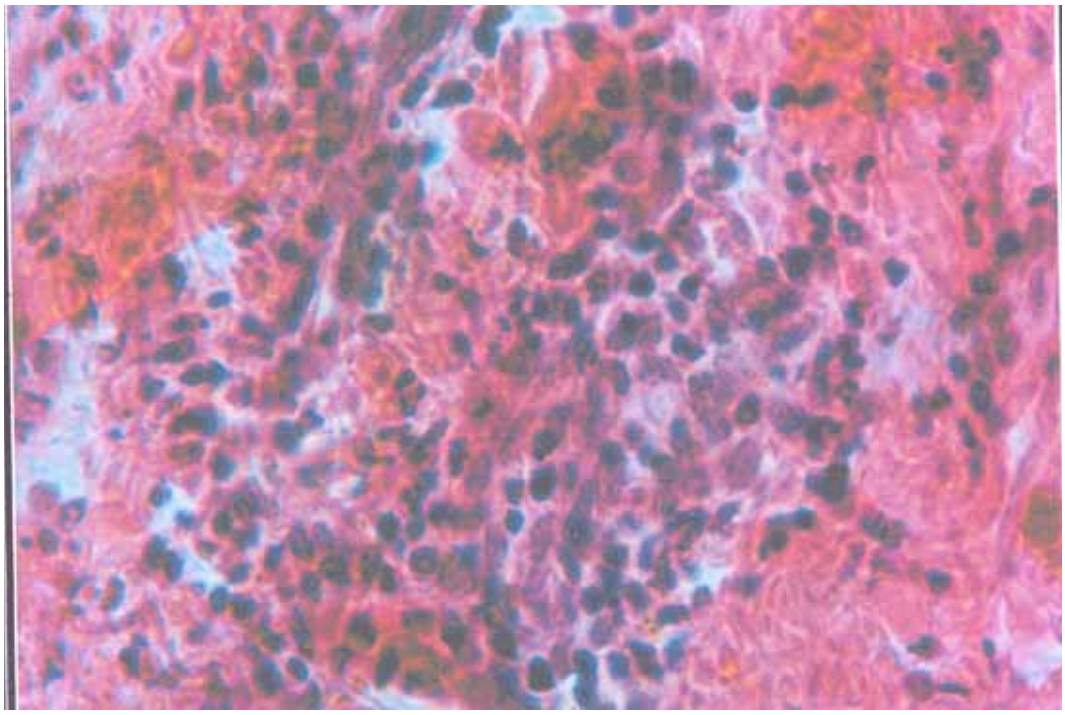


Figure 7: Cross section of a clean open skin wound in sheep at the 15th postoperative day. Many of the crystals of the IPN have disappeared and the rest are degraded and surrounded by foreign-body giant cells. H & E. 160X.

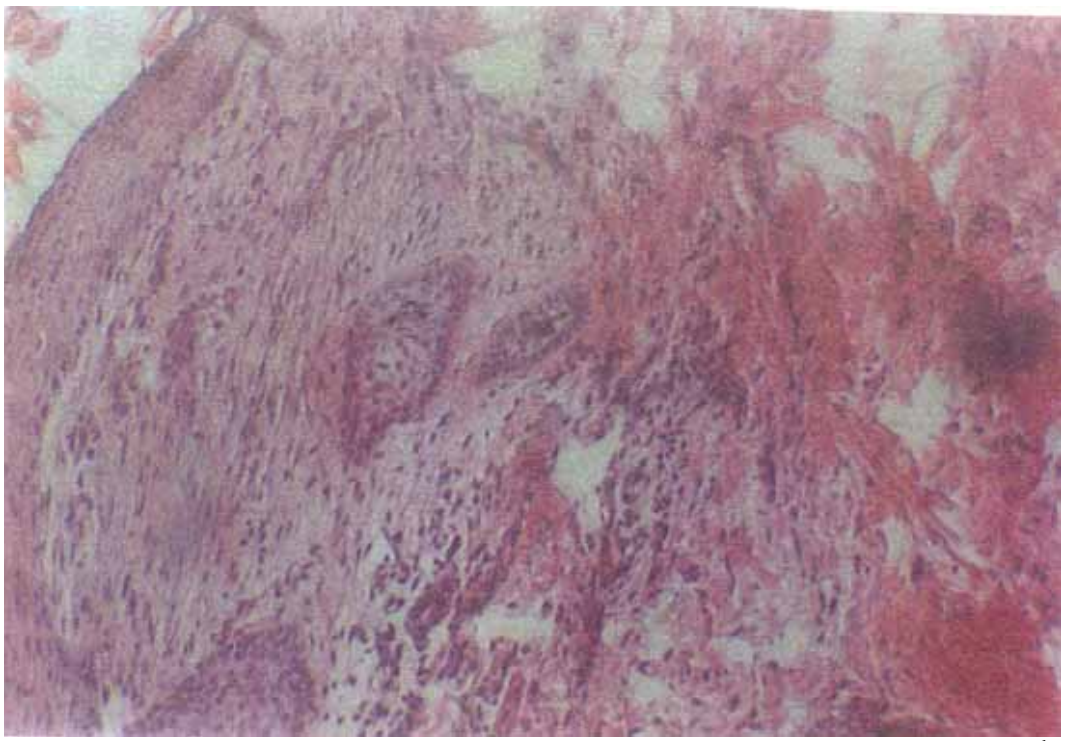


Figure 8: Cross section of a contaminated open skin wound in sheep at the 7th postoperative day. Note a dense mature granulation tissue is filling the wound defect. H & E. 40X.

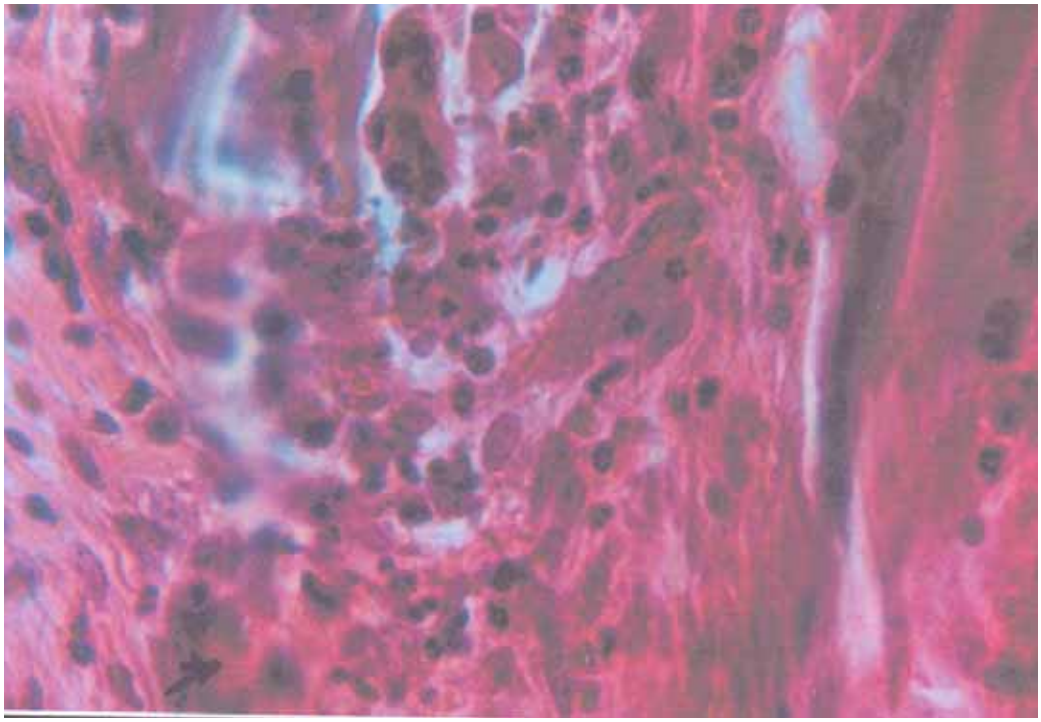


Figure 9: Cross section of a contaminated open skin wound in sheep at the 15th postoperative day. Note the accumulation of mononuclear cells in the granulation tissue and the present of some giant cells around the IPN crystals. H & E. 160X.

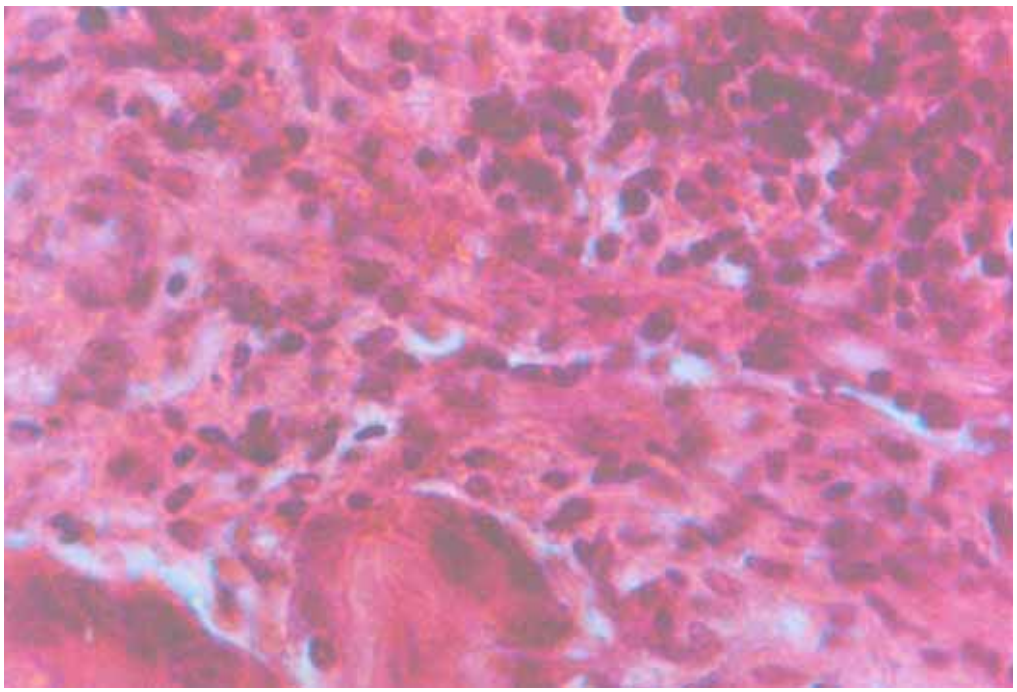


Figure 10: Cross section of a contaminated open skin wound in sheep at the 90th postoperative day. Note the presence of mature granulation tissue infiltrated by mononuclear cells by few giant cells particularly around IPN crystal. H & E. 160X.

DISCUSSION

In the present study, the aims of adding polyurethane to the cross-linked chitosan were to give the chitosan network flexibility and to increase its tensile strength and porosity through the liberation of Co_2. Infrared examination of the IPN was done to ascertain the mechanical properties of the network flexibility, homogenicity, and status of the polymer. This technique has been also used by others and for the same purpose (2, 17). Results of infrared examination of the IPN showed that this network was better than the cross-linked chitosan alone. Chitosan is a biodegradable compound and biodegradation is accomplished through hydrolysis with lipase and chitosanase (32). The 70% of chitosan used in the IPN in this study was chosen due to the good results it gave in wound healing as compared to other concentrations. This finding is in accordance with that of others (33) who also found that using 50% chitosan has lead to disruption of the network and its withdrawal to one of the sides of the wound.

Results of the toxicological pathology in rabbits have shown that the IPN did not have any deleterious effects on the tissues (liver, kidneys, and intestines). This indicates that the IPN was biocompatible. This finding was in agreement with that of other workers (2, 24, 34, 35) and did not differ from that of others who used high dose of chitosan orally for the treatment of inflammations of the urinary tract (15).

As far as measurements of AST, ALT, ALP, and ACP are concerned, significant differences were not encountered between the values of ALT, ALP, and ACP in sheep with clean wounds and in sheep with contaminated wounds. This finding indicates that the IPN did not have any deleterious effect on the liver, kidneys, and the skeletal muscles of sheep with clean wounds and in those with contaminated wounds. However, the values of AST were higher significantly ($P<0.01$) in sheep with infected wounds than in sheep with contaminated wounds. This difference could be due to the fact that AST has the function of transferring the α amine group from aspartic acid to ketonic acid and that the amine groups of the chitosan could be intermingled with those present in aspartic acid. It could be also due to the influence of wound pH since contaminated wounds contain higher quantity of tissue debris than the clean wounds. These findings and postulations were in accordance with those of others (16, 36, 37, 38).

Results of histological examination of the healing clean wounds that were implanted with IPN in sheep were similar to those reported in the literature (15-16, 39-44). However, an additional complication was the presence of the IPN crystals in the healing wounds. These crystals have elicited a mononuclear cell reaction and the encircling of the crystals with foreign-body giant cells. This means that the IPN crystals were inert but still capable of eliciting a foreign –body granulomatous reaction. In clean wounds, the IPN crystals disappeared completely from the healing wounds after the 60^{th} postoperative day. Healing of contaminated wounds occurred in a similar way and at the same rate as that of clean wounds but the IPN crystals disappeared at an earlier period (before the 60^{th} postoperative day). Furthermore, bacterial infection was not encountered in the contaminated wounds. This finding could be explained on the basis of results of previous research findings that chitosan has antimicrobial actions (7, 45, 46). It has been found that chitin-chitosan inhibits in vitro growth of microorganisms including Candida and in vivo has a protective effect on Candida infection (45). When chitosan was dissolved in saline, distilled water, or laboratory media, it exhibits

antimicrobial activity against some strains of filamentous fungi (47, 48), yeasts (47, 49), and bacteria (50). The mode of chitosan antimicrobial action is still obscure (7). That the IPN crystals disappeared earlier from contaminated wounds than from clean wounds could be attributed to the ability of many types of bacteria to produce chitosan-degrading enzymes (chitosanase and lysozyme) (7). Additionally, it has been found that the rate of disappearance of the crystals of chitosan depends on the degree of polymerization, mechanical properties, molecular weight, and structural chemistry of the implant as well as temperature and pH of the medium in which the implant is present (51).

In the present study, the healing of the clean and contaminated open wounds in sheep proceeded with a good rate that coincided with that of clean open wounds (without implant). This finding is in agreement with that of others who found that chitosan has enhancing effects on wound healing (2, 6, 13, 15, 18, 37, 38, 52). These effects could be related to the biocompatibility and biodegradability of the chitosan implant, the hemostatic and antimicrobial actions of chitosan, as well as the enhancement of collagen production and stimulation of fibroblasts by chitosan.

From results of the present study it could be concluded that (1) a cross-linked chitosan- polyurethane mesh was successfully prepared and proved useful in supporting existing tissue or in replacing lost tissue; (2) a 70% chitosan was the most suitable addition to the network to improve its biodegradability and tensile strength; (3) the IPN was non-toxic and could be used safely in animals; (4) chitosan has a beneficial effects on the process of wound healing of both clean and contaminated open wounds; and (5) chitosan has an antibacterial effect in healing wounds.

REFERENCES

1. Dodane V, Vilivalam VD. Pharmaceutical applications of chitosan. PSTT 1998; 1(6): 246-252.
2. Ishihara M. Photocrosslinkable chitosan hydrogel as a wound dressing and a biological adhesive. Trends Glycosci Glycotechnol 2002; 14 (80): 331-341.
3. Sathirakul K, How NC, Steven WF, Chandrkrachang S. Application of chitin and chitosan bandages for wound healing. Adv Chitin Sci 1996; 1: 490-492.
4. Kuthar H, Dawaod S. Preparation of new mesh IPNS (Chitosan-gelatin) to drug delivery of streptomycin. Basrha J Vet Res 2004; 1(1): 1-11.
5. Turner L. Three cheers for chitosan. J Biol Chem 2000; 260: 3041-3049.
6. Ravi-Kumar MN. Chitin and chitosan for versatile applications: An internet chitin and chitosan applied research resource. J Polym 2000; 22: 11-18.
7. Rhoades J, Roller S. Antimicrobial actions of degraded and native chitosan against spoilage organisms in laboratory media and foods. Appl Environ Microbiol 2000; 66(1): 80-86.
8. Kim YH, Choi HM, Yoon JH. Synthesis of a quaternary ammonium derivative of chitosan and its application to a cotton antimicrobial finish. Text Res J 1998; 68: 428-434.

9. Khan TA, Peh KK, Ch'ng HS. Mechanical, bioadhesive strength and biological evaluations of chitosan films for wound dressing. J Pharmaceut Sci 2000; 3(3): 303-311.
10. Hsu W-C, Spilker MH, Yannas IV, Rubin PAD. Inhibition of conjunctival scarring and contraction by a porous collagen-glycosaminoglycan implant. Invest ophthalmol Vis Sci 2000; 41: 2404-2411.
11. Mi FL, Shyu S, Wu Y, Lee S, Shyong JY. Fabrication and characterization of a sponge-like asymmetric chitosan membrane as a wound dresing. J Biomat 2001; 23: 165-173.
12. Chung DR, Kasper DL, Oanzo RJ, Chtinis T. T-cells regulate surgical and post-infections adhesion formation. J Exp Med 2002; 195 (11): 1471-1478.
13. Ghamsari SM, Dehghan MM. Evaluation of tissues and chitosan effects on open wound healing in horses. 5th congress of the veterinary wound healing association held in Hannover, Germany, 2004.
14. Kane R. The design of polymeric multivalent inhibitors of bacterial and viral diseases. J Biomed Mat Res 2003; 27 (11): 1200-1207.
15. Ueno H, Mori T, Fujinaga T. Topical formulations and wound healing applications of chitosan. Drug Deliv Res 2004; 60(20): 105-115.
16. Tramontina VA, Kim SH, Vizzioli MR, Filho GR. Effect of chitosan on wound healing in rats: histological and histometric findings. Braz Dent J 2002; 13(1): 11-16.
17. Tanveer AK, Kok KQ, Hung SC. Mechanical, bioadhesive strength, and biological evaluations of chitosan films for wound healing. J Pharm Pharmaceut Sci 2000; 3(3): 303-311.
18. Poleganova IU, Larumov V, Papdin N. The treatment of suppurative and atonic wounds with chitosan. Khiurgiia, Sofia 2000; 74 (8):27-29.
19. Risbud K. Therapeutic uses of chitosan. Amer J Sci 2004; 34: 351-359.
20. Almada L, McAlindon T. Glucosamine update: nutritional therapies for joint pain. J Biol Chem 2004; 280: 4002-4010.
21. Cottaneo MV, Demierre M. Biodegradable chitosan for topical delivery of retinoic acid. J Biomed Mat Res 2004; 51: 586-590.
22. Liu W, Sun SJ, Zhang X, Yao KD. Self aggregation behavior of alkylated chitosan and its effect on the release of a hydrophilic drug. J Biomat Sci Polym 2003; 14 (8): 851-859.
23. Sendil KD, Altunay H, Wise DL. In vitro pain relief effectiveness of an analgesic anaesthetic carrying biodegradable controlled release red systems. J Biomat Sci Polym Ed, 2003; 14 (6): 497-514.
24. Qujeq D, Ashur MG. Effect of dietary chitosan on nitrogen metabolite levels in mice. Int J Nutr 2004; 30: 90-92.
25. Piyabuter K. Chitin, chitosan and derivatives. 2002. Retrived from Internet: http://www.Iran Polymer Institute.Org.Htm.
26. Granja PL, Silva AI. Preparation and characterization of injectable chitosan hydroxyapatite microspheres. 2003. Retrived from Internet: http://www.Scientific net./A:/ composite.Htm.
27. Nelson JS, Orenstein A, Liaw LH, Berus MW. Mid-infrared erbium-YAG laser ablation of bone and wound healing. Surg Med 1989; 9(4): 362-374.

28. Dealer L. What is UV sterilization? 2003. Retrived from Internet: http://www.emperoraquaties.com/forum//A:/Retialkooiu/Goldfish systems.Htm.
29. Athous DD, Dee KC, Glen AL. Baseline creep characterization of collagen fiber scaffolds. J Sci 2004; 1: 1-7.
30. Kiernan JA. Histological and histochemical methods: theory and practice. 3rd ed. Oxford: Butterworth-Heinemann, 2000: 111-113.
31. Zar JH. Biostatistical analysis. 4th ed, New Jersey: Prentice-Hall Inc. 1999: 283-286.
32. Finer Y, Santerre JP. Biodegradation of a dental composite by esterase: dependence on enzyme concentration and specification. J Biomater Sci Poly 2003; 14(8): 837-849.
33. Dinesh A, Howard WT. Effect of molecular architecture on the mechanical and cell interaction properties of chitosan films. Retrived from Internet (2003): http://www.dineshaggarwad.com/AicHe nonoscale science and Engineering from htmI.
34. Klokkevold PR. The effect of Poly-N-acetyl- glucosaminoglycan (chitosan) on osteogenesis in vitro. Ph.D thesis, Science of oral biology, University of California, 1995.
35. Katalinich M. Characterization of chitosan films for cell culture application. M.Sc. thesis, Marine Science, University of Maine, 2001.
36. Hoffman R, Weiler A, Rehm K. Local foreign-body reaction to biodegradable implants. Unfallhirung 1997; 100: 658-660.
37. Mirzadeh H, Yaghobi N. Polyvinyl alcohol/chitosan blends: preparation, mechanical, and physical properties. Iranian J polym Sci Technol 2002; 11(1): 68-74.
38. Ng KW, Khor HL, Achuth HN, Mox H, Totte SH. The evaluation of natural and synthetic 3D matrices as suitable dermal substrates. J Sci Mat 2002; 73(5): 70-72.
39. Nuutinen JP, Clerc C, Tormala P. Mechanical properties and in vitro degradation of self reinforced radioopaque bioresorbable polylactid fibers. J Biomat Sci Polym 2003; 14 (7): 665-667.
40. Mitchell RN, Cotran RS. Repair, cell regeneration, fibrosis, and wound healing. In: Kumar, V, Cotran R, Robbins SL (eds) Basic pathology. James A. Perkins: Boston, 6th ed, 1997; 47-59.
41. Paul A, Kokate J, Doug S, Keith L, Edward C. Wound formation/treatment of healing. J Clin Engineer 2002; 1(20): 19-26.
42. Regan MC, Barbull A. The cellular biology of wound healing. Verlag, Berlin, Hiedelberg 2001; 135: 1-15.
43. Silver T. Wound repair. 2001, Retrived from Internet: http://www.Advanced tissue.com/about us/01//main/htm1.
44. Singh H, Singh K. Wound healing and tissue repair. In: Singh JI and Tyagi RP (eds) Ruminant surgery. Shadrar-Delhi: CBS Publishers and Distributors, 1996: 58-71.
45. Koide SS. Chitin-Chitosan: Properties, benefits and risks. Nut Res 1998; 18(60): 1091-1099.
46. Vesil F. Biodegradable wound dressing improves healing. PDC Newsletter 2000; 1(2): 20-26.

47. Kendra DF, Hadwiger LA. Characterization of the smallest chitosan oligomer that is maximally antifungal to Fusorium solani and elicits pisatin formation in Pisum sativum. Exp. Mycol 1984; 8: 276-281.
48. Roller S , Covill N. The antifungal properties of chitosan in laboratory media and apple juice. Int J Food Microbiol 1999; 135: 67-77.
49. Sundarshan NR, Hoover DG, Knorr D. Antibacterial action of chitosan. Food Biotechnol 1992; 6: 257-272.
50. Wang G-H. Inhibition and inactivation of five species of foodborne pathogens by chitosan. J Food Prot 1992; 55: 916-919.
51. Bug M. Artificial skin dressing materials and noval delivery systems. http://www.purapet.com., 2003.
52. Mathes SJ , McGrath MH , Nance FC, O'leary JP. Symposium: Abdominal wall reconstruction. Contemp Surg 1999; 54(4): 238-249.