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Effects of fish scales extracted collagen biocasings on cutaneous wound healing in dogs.

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ABSTRACT

Dogs with cutaneous wound were selected and randomly divided into two groups, to study the efficacy of fish scales extracted collagen biocasings on cutaneous wound healing. The wounds in both groups of dogs were protected with sterile external bandage after cleaning with normal saline to prevent infection. The efficacy of biocasings on wound healing was studied based on gross, physiological, hematological, biochemical and histological findings at different time intervals of observation. The changes in gross, physiological, biochemical and hematological parameters were due to stress in control group and due to presence of foreign body reaction in biocasings applied group. The histological sequences such as inflammatory reaction, edema, fibroblast proliferation, collagen synthesis, capillary vascularization and re epithelialization were more evident in collagen biocasings applied wounds. Fish scales extracted collagen biocasing did not allow accumulation of wound fluid compared to non applied wounds. Healing time was significantly low in collagen biocasing applied wounds (15.00±0.82 days) compared to untreated group (23.67±0.99 days). The fish scales extracted biocasings were moderately adherent to wound surface but produced good haemostatic effect and resorption was evident in 48 hrs of post application. The biocasings applied wounds gained nearly normal tensile strength at 28th post operative day. The use of fish scales extracted collagen biocasings for cutaneous wound healing in dogs was effective and helped in augmentation of wound healing activity.
Key words: Fish scales extracted collagen, cutaneous wound healing, dogs, gross studies, histopathology

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INTRODUCTION

Management of traumatized wounds in dogs is a big task in veterinary practice and are accompanied by hemorrhage, contamination and infection. The complications of wound healing are attributed to defective basic repair process such as inadequate formation of granulation tissue, deficient scar formation and excessive formation of repair components or formation of contracture. Various scientists tried by homograft, allografts, xenografts [1] to treat traumatized wounds and burns in human patients. However these grafts had limitations in use such as poor survival rate, difficult to harvest and do not provide the enough strength and cosmetic quality [2].

All the traumatized wounds in dogs are unsuitable for closure by traditional method of suturing, but by using pinch graft good results are observed in graft with good hemostasis and better conditions for healing [3] But their use is restricted due to the inadequate availability and difficulty in storage of skin grafts hence the concept of use of biomaterials for wound healing came into existence as the biomaterials possess the characteristics such as non-irritant, nontoxic, non inflammatory to neighbouring tissue and non antigenic in nature. The biomaterials possessease of adherence which provide better hemostasis, accelerate phagocytes, early epithelialization and revascularization. Collagen is a biologically active and very dynamic wound healing material applied on to wound bed, its matrix creates the proper environment for the skin tissue and for cellular activity which is necessary for healing process.

Collagen activates inflammatory phagocytic cells and increases vascularization of the repairing tissue by tri-dimensional structure of the collagen protein molecule with a unique ability to elicit growth of the fibroblastic network which is important for the granulation tissue formation [4]. Collagen is the most commonly available protein from animal source providing extra cellular matrix and collagen of different species such as calf, rat, human, carp and sea anemone have same structure. By considering the above properties, an attempt was made to study the efficacy of collagen biocasings in treating cutaneous wounds where the collagen involved in present study was extracted from fish scales and applied on cutaneous wounds in clinical cases of dogs.

MATERIALS AND METHODS

Preparation of collagen biocasing extracted from fish scales

Fish scales were collected from the market and purified. The scales after purification were subjected to controlled alkaline hydrolysis and brought to neutral PH. Later the scales were subjected to acid hydrolysis for 24 hours. The extracted collagen was made in to sheet form and dried. The dried sheets were cut in to required size, sterilized with ethylene oxide for 3-4 hours and stored.

Wound treatment

24 apparently healthy dogs presented to the hospital with cutaneous wounds were randomly selected for the present study. All the dogs were dewormed with IVERMECTIN* at the dose of 0.02 mg / kg body weight, given subcutaneously prior to study. The dogs were divided in to two groups each of 12 dogs to study the efficacy of collagen extracted from fish scales on wounds of 3.5X3.5cm area. 12 dogs with cutaneous wounds were randomly selected as Group I – control animals and the wounds were converted in to 3.5 X 3.5 cm size, was not closed and left as it was. The wounds were cleaned with normal saline and covered with external bandage to prevent auto mutilation and contamination. The same procedure was carried out every alternate day till the wound showed healing. In group II, in addition to normal saline cleaning, collagen biocasings extracted from fish scales were applied over the cutaneous wounds. Collagen biocasings of corresponding uniform size were snugly applied after making few pores with sterile needle of 20G to have better adhering property over the wound without loosening or separation. The wounds were protected by using external bandage. The bandaging was changed once in two days which provided immobilization to the wound edges during healing in addition to protection. [5, 6]. The protective gauge bandage covering the site was replaced once in two days by a fresh one.All the parameters were recorded in following studies on the day of Zero, 7^{th} , $14^{th}$and 28^{th} of post treatment.

Gross studies

The wound healing was studied clinically at regular intervals for the extent of cicatrisation and for determination of the area of wound healing in both groups[7].

Physiological studies

Different physiological parameters were recorded on specified days after treatment. (i)The rectal temperature was recorded using electronic digital thermometer and expressed as °F. (ii)The respiratory rate was noted and expressed by observing the thoraco abdominal movements during inspiration and expiration as breaths per minute. (iii) Pulse rate was recorded by palpation of femoral artery and expressed as rate per minute

Haematological studies

Five milliliters of venous blood was collected from the cephalous vein in ethylene diamine tetra acetic acid (EDTA) added sterilised vials for estimation of different haematological parameters at specified days of post treatment . These were estimated with Automated Haematology Analyser–DIAMS 20V* (Ph – Diagnostics) following standard procedure [8]. The parameters estimated by autoanalyser were compared with manual methods of analysis for correlation. The hematological parameters estimated were total erythrocyte (millions/cmm.), total leucocyte count (thousands/cmm.), haemoglobin content (gm%), packed cell volume (%) and differential leucocyte count(%). All the data were subjected to paired t- test and RBD [9].

Histological studies

Biopsy specimen from the wound site of all dogs was collected by trephining the skin piece into 10% buffered fomalin at specified intervals and processed by routine paraffin embedding technique. 5µ thick sections were cut and stained with haematoxylene and eosin. [10]. Van-Gieson's method of staining was used for the demonstration of collagen fibers in sections. [11].

Biochemical studies

The blood samples were collected from all the dogs at specified days and the changes in different biochemical parameters were recorded by using *star 21 and auto analyzer. The serum glucose levels (mg/dl) was estimated by Teitz method of glucose oxidase- peroxidase (GOD-POD) [12], serum total protein levels (gm/dl) [13,14], alkaline phosphatase levels (I.U./L) [15], acid phosphatase levels (I.U./L) [16] and C-reactive protein levels (mg/L) were also estimated by latex agglutination kit method. [17,18].

Estimation of tensile strength

The tensile strength of group II was compared with normal skin by measurement of maximum load (nutons) , extension of maximum load (mm.)and tensile strength maximum load (milli pounds and %).

Statistical analysis

All the data were subjected to paired t-test and RBD test [9] to find statistical differences between two groups.

RESULTS

The present study was aimed to evaluate the efficacy of collagen (extracted from fish scales) biocasings on cutaneous wound healing in dogs. After application of biocasings various gross, physiological, hematological, biochemical and histological studies were conducted.

Gross studies

Gross findings were recorded at specified days after treatment. Group I dogs showed wound fluid on 7^{th} day whereas the wound fluid was absent in group II dogs. The biocasings were intact, well adhered to the wound surface and were absorbed by 3^{rd} post operative day. Locally there was no inflammatory edema and exudates in group II dogs. In both groups wounds were protected externally using steripad dressing to prevent the external contamination and auto mutilation. None of the animals in both groups showed signs of infection. Complete healing was observed within 23.67± 0.99 days in group I animals where as group II took 15±0.82 days with a significant difference ($P<0.01$) between the groups (fig.1and

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2). The percentage of wound contraction at regular intervals was significantly different in two groups (P<0.01) as given table no.1.

Table 1:Mean±SE values of wound contraction, physiological parameters at different days in both groups

Name of the parameter (units)	Days							
	0		7		14		28	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
Wound contraction (%)	-	-	22.86[a]± 1.28	30.00[b]± 1.77	45.72[a]± 1.47	**62.86[b]± 3.04	73.81[a]±1. 15	**99.99[b] ±0.00
Physiological properties								
Temperature (^{0}F)	101.43 ±0.31	101.50± 0.38	101.93± 0.22	101.40± 0.38	*102.30± 0.57	101.37± 0.33	101.33±0 .28	101.53± 0.37
Respiratory rate (breaths/mt.)	24.33± 1.20	25.67± 1.05	*27.33± 0.67	24.50± 1.09	26.00± 0.73	24.33± 0.95	23.17 ±0.75	23.33 ± 0.33
Pulse rate (beats/mt.)	104.5± 1.41	104.0± 2.38	107.33± .99	109.50± 1.54	107.83± 1.97	101.33± 4.31	106.00±1 .24	108.33 ± 1.89

*Significant at (P<0.05);**Significant at (P<0.01); Group I : Control group; Group II : Biocasings applied group Means with superscripts a & b differ significantly at (P<0.01)

Physiological studies

The changes in physiological parameters like temperature, respiration, pulse rate are shown in table no. 1.

Temperature

The temperature values showed a significant difference (P<0.05) between the groups on 14th day. The values in group I showed a significant increase (P<0.05) within the maximum of 102.30±0.57°F on 14th day, thereafter the values were reduced. Group II animals showed decrease in values upto 14th day followed by mild non-significant increase. The values reached nearly pre-mean levels by 28th post treatment day in both the groups. All the fluctuations were within the normal physiological range.

Respiratory rate

The values showed significant (P<0.05) increase up to 27.33±0.67 on 7th day followed by decrease up to 28th day, whereas group II animals showed a non-significant fall reaching a minimum of 23.33±0.33 on 28th day. No significant difference was observed between the groups at any period of observation.

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Pulse rate

The pre-mean pulse rates recorded were 104.50± 1.41 and 104.0±2.38 in group I and II respectively. Group I animals showed non-significant increase up to 14th day followed by non-significant fall. Group II animals showed non-significant increase to 109.5±1.54 thereafter, a non-significant fall or rise during the rest of periods. No significant differences were observed at any period of observation between the groups during the study. The values were fluctuated within the normal physiological limits.

Haematological studies

The alterations in hematological parameters are shown in table no.2.

Table 2 : Mean± SE values of haematological parameters at different days in both groups

Name of the parameter (units)	Days							
	0		7		14		28	
	Group-I	Group-II	Group-I	Group-II	Group-I	Group-II	Group-I	Group-II
Total erythrocyte count (millions/ mm^3)	6.53±0.46	7.27±0.85	7.02±0.46	7.21±0.76	7.18±0.26	7.05±0.21	7.20±0.21	7.24±0.21
Total leukocyte count (millions/ mm^3)	14.6±1.68	12.65±1.19	9.58±1.10	9.37±0.91	8.98±1.87	10.62±0.68	12.47±0.82	13.15±1.16
Hemoglobin (%)	13.08±0.49	13.88±0.43	13.17±0.75	13.30±0.50	13.3±0.58	13.15±0.47	13.25±0.41	13.87±0.59
Packed cell volume (%)	39.28±1.49	41.93±1.32	38.98±2.21	41.48±1.16	39.82±1.71	39.47±1.40	39.78±1.24	42.67±2.06
Neutrophils (%)	63.00 ± 2.86	71.00 ± 2.16	69.00 ± 1.63	61.33 ±2.99	66.83 ± 2.23	67.33 ± 2.25	64.33 ±1.15	66.00 ± 1.63
Lymphocytes (%)	22.17 ± 2.98	11.83 ± 2.18	20.33 ± 1.31	22.67 ± .61	20.67 ± 2.79	16.67 ± 2.59	24.30 ± 0.84	22.33 ± 2.69
Esinophils (%)	11.33 ± 0.67	10.50 ± 1.38	03.67 ± 1.73	**11.17 ± 2.02	10.00 ± 0.73	09.67 ± 2.26	**10.67 ± 0.99	08.33 ± 1.12

*Significant at (P<0.05);**Significant at (P<0.01); Group I : Control group; Group II : Biocasings applied group
Means with superscripts a & b differ significantly at (P<0.01)

Total erythrocyte count: Group I animals showed a non-significant rise in total erythrocyte count and reaching a maximum of 7.2±0.21 at 28th day, whereas group II animals experienced a non-significant fall to minimum of 7.05±0.21 on 14th day followed by a non-significant rise.

Total leucocyte count: The total leucocyte counts in group I and II were 14.67±1.68 and 12.65±1.19 thousands/mm^3 respectively. The total leucocyte count in group I animals showed

a non-significant fall up to 14th day followed by the significant increase (P<0.01) on 28th day, whereas group II animals showed a significant fall (P<0.01) reaching a minimum of 9.3±0.91 thereafter the values tended to increase reaching nearly normal values on 28th post operative day.

Haemoglobin: The pre mean values were 13.08±0.49 gms % in group I and 13.88±0.43 gms % in group II dogs.

Packed cell volume: Both groups of animals showed non-significant decrease in values up to 7th post operative day in group I and up to 14th day in group II. Thereafter the values showed a tendency to increase non-significantly.

Differential leucocyte count: A non-significant initial rise was observed in control dogs at 7th post operative day followed by a gradual non-significant fall. Group II animals showed a significant fall (P<0.01) in neutrophil counts at 7th day, thereafter the values tended to rise till last observation. The lymphocyte counts in group I experienced a non-significant lymphocytopenia up to 14th day, thereafter showed a non-significant rise contrary to this, group II animals showed initial significant lymphocytosis (P<0.01) followed by a gradual fall to a minimum of 16.67±2.59 at 14th day. The pre-mean eosinophil, monocytes and basophil counts were 11.33±0.67 and 10.50±1.38, 1.17±0.65 and 2.33±0.61 and 2.33±0.76 and 4.33±0.84 in group I and II respectively. No significant difference in hematological parameters was noticed between two groups at any period of observations and however the fluctuations were within normal physiological range.

Histological studies: The wound in control group showed mild infiltration of mono nuclear cells with moderate proliferation of fibrous tissue whereas the wound in group II animals showed three distinct layers of skin, with homogenous pinkish mass and infiltratory cells, supported by proliferated fibrous tissue on 7th day. The histological examination of wounds of control group dogs on 14th day showed abundant fibrous tissue proliferation with very few mononuclear cells in the stratum germinativum. The fibrous tissue was parallelly arranged to stratum cornium and capillaries were emerged in stratum germinativum The biocasings applied wounds showed marked fibrous tissue proliferation with more vascularisation. The fibrous tissue was arranged perpendicular to stratum cornium. On twenty eighth day, the wound in control group showed abundant fibrous tissue proliferation with increase in thickness of stratum leucidum, there were numerous capillaries and showed epithelialization (fig.3).The biocasings applied wounds showed abundant vascularization, the epithelium was almost normal skin appearance with three distinct layers. Linear collagen fibres could be evident of remodeling (fig.4)

Biochemical studies: The alterations in biochemical parameters are shown in table no 3.

Glucose: Glucose values in group I showed non-significant decrease followed by a non-significant rise up to 28th post operative day reaching a maximum of 89.63±3.49 mg/dl, whereas the fall was significant (P<0.05) in group II on 7th post operative day reaching a minimum of 92.05±3.65 thereafter the values reached nearly base level values at 28th day of observation.

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There was a significant difference between two groups in glucose levels (P<0.01) at 14th and 28th days of observation.

Table 3 : Mean±SE values of biochemical parameters at different days in both groups

Days								
Name of the parameter (units)	0		7		14		28	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
Glucose (mg/dl)	88.75±4.25	*97.07±3.40	86.30±3.24	92.05±3.65	87.10±3.18	**94.83±3.25	89.63±3.49	*97.28±3.11
Total protein (gm/dl)	6.47±0.47	6.87±0.37	5.22±0.39	5.74±0.24	6.91±0.65	7.34±0.56	6.30±0.25	7.20±0.39
Alkaline phosphatase (I.U)	20.15±2.72	11.56±1.60	31.57^{a}±2.05	**16.99^{b}±3.34	27.37^{a}±2.12	**13.65^{b}±1.93.	21.72^{a}±2.48	**11.67^{b}±1.59
Acid phosphatase (I.U)	5.42±0.91	2.17±1.04	5.89^{a}±0.60	**2.82^{b}±1.11	5.08^{a}±0.73	**2.48^{b}±1.14	4.45^{a}±0.43	**2.29^{b}±1.09

*Significant at (P<0.05);**Significant at (P<0.01);Group I : Control group; Group II : Biocasings applied group
Means with superscripts a & b differ significantly at (P<0.01)

Total protein: Both the groups showed non-significant decrease in total protein levels, reaching a minimum of 5.22±0.39 in group I and 5.74±0.24 in group II at 7th day of observation later the values raised non-significantly during the rest of the periods of observation. Both group I and II animals showed significant decrease (P<0.05) on 7th day and increase on 14th day. There was no significant difference between the groups at any period of observation.

Alkaline phosphatase: There was a significant difference (P<0.05)in alkaline phosphatase levels between two groups on 7th day. The group I and II animals showed significant (P<0.01) rise on 7th post operative day reaching a maximum of 31.57±2.05 and 16.99±3.34 respectively. Thereafter both the groups showed a decrease in tendency reaching nearly pre-treatment values at 28th post operative day.

Acid phosphatase: There was non-significant increase in the values to the maximum of 5.89±0.60 in group I animals on 7th day, thereafter the values showed a significant decrease (P<0.01) on 28th day, whereas group II animals showed a non-significant increase on 7th day with a non-significant fall to minimum of 2.29±1.09 on 28th day. There was a significant difference (P<0.05) in acid phosphatase levels between the groups on 7th and 28th days.

C-reactive protein: C-reactive protein levels in serum were tested with latex agglutination test which gives positive reaction when the levels of C-reactive protein exceeds 6 mg/L. During the entire period of study none of the dogs in both groups revealed positive reaction with latex

agglutination test which was an indication that the C-reactive protein levels remained in normal range throughout the period of study and there were no significant alterations recorded.

Tensile strength estimation: The tensile strength parameters in samples of normal skin and skin with biocasings at 28th day post application were studied and shown in table no.4. Normal skin showed a maximum load of 30.29 nutons, extension of maximum load 9.67 mm, tensile strength maximum load 1.99 mpa and 54.44%, where the biocasings applied sample showed maximum load 9.71 nutons, extension of maximum load 9.67 mm., tensile strength maximum load 0.97 mpa and 54.44%. There was a significant difference (P<0.01) between normal and biocasings groups with respect to maximum load and tensile strength maximum load

Table 4: Tensile strength parameters of normal skin and skin of wound healed by biocasings at the end of study

Sl. No.	Tensile strength parameters	Normal skin	Biocasings applied wound
1.	Maximum load (nutons)	**30.29^{a}	9.71^{b}
2.	Extension of maximum load (mm)	9.67	9.67
3.	Tensile strength maximum load (milli pounds)	**1.99^{a}	0.97^{b}
4.	Tensile strength maximum load in percentage (%)	54.44	54.44

*Significant at (P<0.05);**Significant at (P<0.01);Group I : Control group; Group II : Biocasings applied group
Means with superscripts a & b differ significantly at (P<0.01)

DISCUSSION

The wound treatment procedure was common in both the groups for uniform evaluation. More amount of wound fluid and blood clots were observed in control group dogs which might be due to hemorrhage. Biocasing treated wounds showed neither hemorrhage nor adverse inflammatory reaction by the host tissue, which could be attributed to haemostatic, non immunogenic and sealant activity of collagen biocasings as observed with fibrin glue [19]. The collagen biomaterial was found to adhere to wound surface satisfactorily following its application. None of the animals showed host rejection or biocasings separation or any other gross changes. The adhering property of collagen might be due to the property of promoting platelet aggregation and clot formation [20]. Infection is the major threat cause delayed wound healing, results in to tissue decantation, wound dehiscence and increased morbidity [21].In the present study collagen biocasings showed hydrocolloidal activity, prevented contamination, provided sterile environment and enhanced fibrogenesis, cellular infiltration and collagen deposition (fig.1 and 2).

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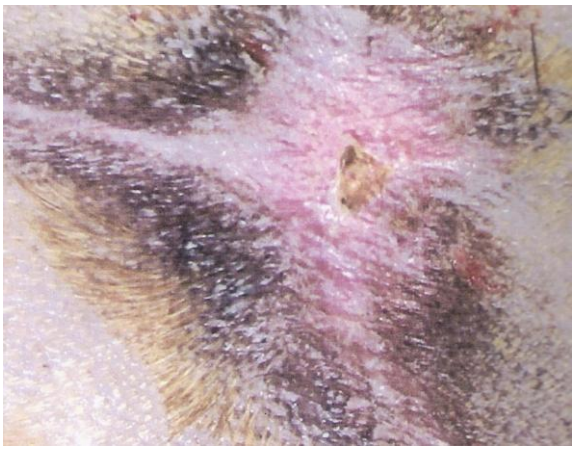

Fig 1 photograph of wound healing in control group on 28 th day

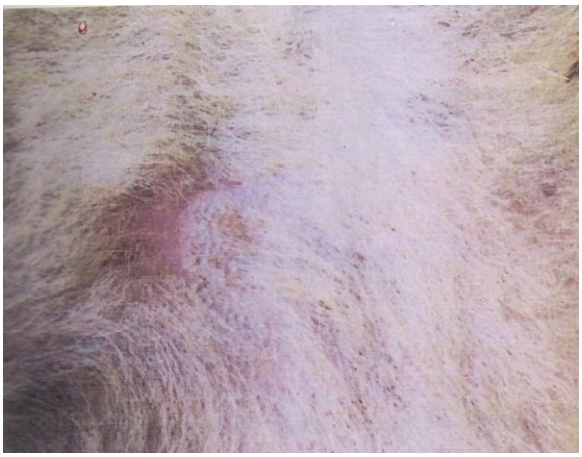

fig 2 photograph of wound healing in biocasings applied group on 28 th day

The hydrophilic property of collagen dressing would clean the contaminated wounds with body's haemostatic fluids [2] which enhance the wound healing process. None of the animals showed infection/sepsis similar to observations observed with hydrolyzed collagen and bioactive collagen sponge as in previous reports[2,22] also reported better wound contraction and early wound healing following biocasings application, probably because of non-toxic nature, minimal tissue reaction properties and similar antigenic determinants of collagen of animals and sea animon [6]. The total erythrocyte counts showed a significant increase in group I on seventh day of observation and no change in group II. The packed cell volume values showed non-significant fall on seventh day in control group and in biocasings applied group non-significant rise to reach base level. The alterations in total erythrocyte count were due to initial stress in control dogs [23,24]. The fall in packed cell volume might be due to mild foreign body reaction elicited by collagen biocasing in treated group dogs [25]. The subsequent return of erythrocyte count and packed cell volume are indicative of well tolerance of biocasings by the host tissue. The total leucocyte counts showed significant fall in both groups of dogs with higher values in biocasings applied group on seventh day of post treatment. The count showed down on fourteenth day of post treatment in control group dogs however biocasings applied dogs showed an increase in total leucocyte count. In both the groups the values reached pre-treatment level by twenty eighth day. The rise in total leucocyte count on the fourteenth day post treatment after a fall as seventh day post treatment might be due to the increased cellular infiltration of collagen and reduced phagocytosis. [22]

Correspondingly non significant neutrophilia was observed among control group dogs and there was significant neutropenia in collagen biocasings applied dogs on seventh day of post treatment with significant rise as subsequent observations but remained at lower till the last observation. There was non significant lymphocytopenia in control group dogs during the early observations which returned to pre-treatment level at the end of study. Biocasings applied group dogs showed significant lymphocytosis throughout the period of study. The eosinophilic count showed significant eosinopenia in control group dogs on seventh day and reached almost pre-treatment level on fourteenth and twenty eighth day. The biocasings

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applied group showed mild eosinophilia on seventh day followed by eosinopenia throughout the period of study. The neutrophilic count alterations are due to early stress in control group dogs and foreign body reaction in biocasings applied group dogs. The variations of lymphocyte counts are suggestive of immune mediated reaction in biocasings applied group of dogs. The non-significant rise of eosinophilic count in biocasings applied group might be due to early physiological stress. These alterations in counts of neutrophil, lymphocyte and eosinophils [26,27,28] and the neutrophelia in control group of dogs was due to chemotactical alterations by presence of devitalized tissue [29].

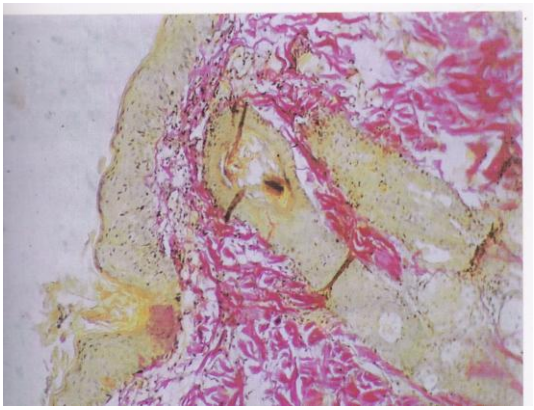

fig 3 photomicrograph of wound on 28 th day in control group

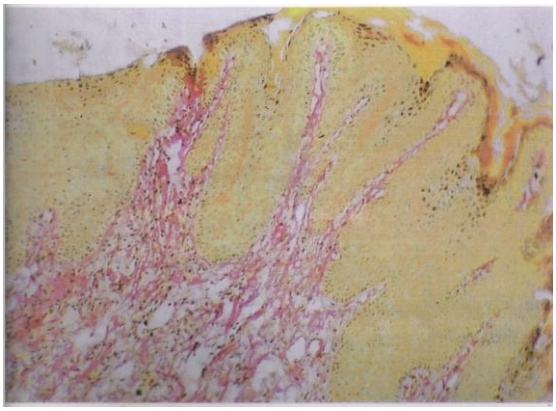

fig 4 photomicrograph of wound on 28th day in biocasings applied group

The microscopic observation of histology is very important in assessing the efficacy and compatibility of biocasings. On the seventh day post treatment the control group dogs showed mild infiltration of mononuclear cells with moderate proliferation of fibrous tissues suggested that there was mild inflammation and collagen synthesis was present as shown in fig.3 The biocasings applied wounds showed three distinct layers with markedly proliferated connective tissue and homogenous pinkish mass with infiltration of few inflammatory cells in fig.4. The findings were suggestive of the biocasing were well tolerated and enhanced collagen synthesis than non biocasing applied wounds as reported by Ansari et al who found similarly with Clendula officinalis ointment, Charmil and Gelatin granules on wound healing in buffaloes [30]. On fourteenth day the control group dogs showed abundant fibrous tissue proliferation with few mononuclear cells and there were emerging capillaries. The biocasings applied wounds showed formation of the distinct layers of skin with more pronounced vascularization perpendicularly arranged linear collagen fibers and more number of mononuclear cells. The proliferating connective tissue was more evident. These findings revealed that the biocasings applied wounds showed better healing process and the biocasings acted as scaffold for wound healing process which is supported by other biocasings like chitosan, collagen enriched with fibronectin and misoprostol [30-33]. The observations on 28th day of post treatment in control group dogs showed abundant fibrous tissue proliferation with increased thickness of stratum lucidum and prominent more vascularization. The biocasings applied wounds showed abundant vascularization and epithelial formation, which almost appeared as normal skin with more prominent linear fibers of collagen. It confirms the completed healing process in

biocasings applied group where collagen fibres are undergoing remodeling compared with control group [30, 34, 35]. The total protein, glucose levels in both the groups showed a transient significant fall on the seventh day followed by rise during the later period of observations however fluctuated within physiological range similar to the findings observed in dogs treated with fibrin, gelatin and amniotic membrane for urethral wound healing [6].

Alkaline phoshpatase and acid phosphatase levels during the present study showed significant variations between the groups with higher values in group I. The alkaline phosphatase plays a vital role in creating alkaline environment at wound site which is essential for fibro plastic activity to synthesize collagen whereas acid phosphatase will cause acidic environment to control over synthesis of collagen and initiates the process of remodeling of collagen. The values of alkaline phosphatase were significantly increased on the seventh day with higher values in control group then showed gradual decrease to pre-treatment level by twenty eighth day. The acid phosphatase levels showed same tendency as alkaline phosphatase with non-significant alterations in both groups, with higher values in control group dogs but significant lower values on twenty eighth day. The initial increase in alkaline phosphatase activity in control group might be to provide alkaline medium for favorable synthesis of collagen in wound area whereas the biocasings applied group showed lower levels than control because of free availability of collagen and the improved cellular infiltration activity of collagen as observed with bioactive collagen sponges [22]. The increased alkaline phosphatase activity would be attributed to increased fibroblastic activity [36,37].

C-reactive protein is a tool for evaluating the health status and is an acute phase protein of infection and inflammation. The levels of C-reactive protein increases as early as four hours after major surgery and the normal dogs contains less than 5 mg/L of C-reactive protein in the serum [38]. Any change in C-reactive protein levels correlates the presence of stress and inflammation. During the present study the C-reactive protein levels in both groups of dogs remained at base level and there was no significant alterations, which was suggestive of no significant inflammatory process in both the groups of dogs at any time interval of observation, though the biocasings were applied as a foreign material. The C-reactive protein regulates the inflammatory process and acts in microbial defense through a variety of compliment.

The parameters of tensile strength estimation such as extension of maximum load and percentage of tensile strength maximum load were same in both normal skin and biomaterial applied wound. There was significant difference between the groups in maximum load and tensile strength maximum load. These variations in maximum load and tensile strength maximum load and similarities of extension of maximum load and tensile strength maximum load in percentage were suggestive that the wound breaking strength and tensile strength maximum load in percentage gained normal level by 28^{th} post treatment day in biocasings applied group with lower values, attributed that the collagen content of the biocasings treated wound is similar to that of normal wound and remodeling is in progress. These findings are in correlation with the observations found by Scott et. al [39]. and Doillon et. al [40,41].

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CONCLUSIONS

The biocasings applied wounds showed neither hemorrhage nor adverse inflammatory reaction by the host tissue with better wound contraction, early wound healing with increased cellular infiltration of collagen and reduced phagocytosis. Biocasings were well tolerated and enhanced collagen synthesis with abundant vascularization and epithelial formation. The use of fish scales extracted collagen biocasings for cutaneous wound healing in dogs was effective and helped in augmentation of wound healing activity

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