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Biochemical and histopathological changes in the treatment of chronic cutaneous wound in New Zealand white rabbits with bio-ceramic device

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Abstract
In this study, 18 (eighteen) numbers of adult rabbits of either sex of New Zealand White maintained under proper managerial condition in the Department of Surgery & Radiology, College of Veterinary Science, AAU, Khanapara were used to produce surgical wounds. The animals were randomly divided into three (3) groups i.e. A, B and C with each group consisting of six (6) animals. The biochemical changes were recorded on 3rd, 6th and 9th day but the histopathological changes were recorded on 9th day. The surgical sites at the thoraco-lumber region were prepared by clipping and application of antiseptic solution (Povidone iodine).The Protein content, Alkaline phosphatase, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Creatine kinase (CK) were determined and estimated by collecting 50 mg of wet tissue from each wound area. The biopsy materials from the wounds of 4mm thickness on 9th day post wound creation were collected and preserved in normal buffered formalin. The maximum protein level of wound tissue homogenate was recorded on 9th day of experiment in all the groups. There was an initial rise of alkaline phosphatase activity in wound tissue homogenate on 3rd day in all the groups. There was initial rise of creatine kinase activity in the wound tissue homogenate in all the groups on 3rd day observation. Thereafter, the creatine kinase activity in the wound tissue homogenate was declining towards the end of the experiment. The histopathological examination of the healing tissue taken on 9th day in Group B revealed thin layer of epidermal cells with a thin keratin layer without any scab. In Group A, some scab materials were still present on the wound surface on 9th day with a thin layer of squamous epithelial cells covering the wound surface. The data were analyzed as per methods described by Snedecor and Cochran (1994) and were presented accordingly.

Keywords: Biochemical, histopathological, wound, bio ceramic, rabbits

1. Introduction
In animal subjects such wounds reduces the economic value and also their working efficiency. The owner loses interest in their pets and from a loveable thing it changes to a nuisance. The surgeons are known as wound Healers. They are concerned with earliest healing of wounds. In present day context the surgeons are also working on various innovative concepts like use of hormones and other substances effecting healing of wound for the fact that the mixed colonies of bacteria in a chronic wound cannot be controlled easily and can result in the development of more resistant bacterial colonies. Bio-Ceramic, a micro porous ceramic that expels off excess wound discharge (fluid produced in the wound) and lock it into the ceramic by high capillary suction power. Bio-Ceramic when tested on various types of acute and chronic wounds showed quick deodorization of wound and signs of healing on continuous usage. The present investigation was undertaken to evaluate the wound healing capacity of Bio-Ceramic Wound Treatment Device by biochemical and enzymatic analysis of treated wound and the tissue level changes following the use of the Bio-Ceramic Wound Treatment Device microscopically.

Materials and Methods
In the present study 18 (eighteen) numbers of adult rabbits of either sex of New Zealand white maintained under identical managerial and environmental condition at the Department of Surgery & Radiology, College of Veterinary Science, AAU, Khanapara were used to produce surgical wounds. The animals were randomly divided into three (3) groups i.e. A, B and C with each group consisting of six (6) animals.

The animals were prepared for surgrical procedure to create the wound by withdrawing food for 12 (twelve) hours and water for 6 (six) hours. The surgical sites at the thoraco-lumber region were prepared by clipping and application of antiseptic solution (Povidone iodine). The treatment protocols were applied after 72 hours of creation of wound to each groups containing equal number of rabbits of either sex under identical managerial and environmental condition (Table 1).

The Protein content, Alkaline phosphatase, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Creatine kinase (CK) were determined and estimated by collecting 50 mg of wet tissue from each wound area in separate sterile tubes containing 0.15 M Potassium chloride (KCl) solution. The tissues were then subjected to homogenization at 7000 rpm for 2 minutes in the same solution @10mg/ml in a Polytron Kinematic, Switzerland homogenizer. The homogenized samples were centrifuged at 4000 rpm for 10 minutes and supernatant were analyzed on the same day. The biochemical changes were observed on 3rd, 6th and 9th day of the treatment.

For the purpose of study, the biopsy materials from the wounds of 4mm thickness on 9th day post wound creation were collected and preserved in normal buffered formalin. After proper and adequate fixation the tissues were processed as per methods described by Luna (1968) [10]. The paraffin embedded tissues were cut into section of 4-5 µ thickness and stained with Haematoxylin and Eosin (H&E) stains (Culling, 1974) [4]. The stained tissue sections were finally examined under the microscope and the histological changes were recorded.

The data were analyzed as per methods described by Snedecor and Cochran (1994) [16]. and were presented accordingly.

Results

The maximum protein level of wound tissue homogenate was recorded on 9th day of experiment in all the groups. The rate of increasing trend in protein level of wound tissue homogenate as shown in was observed rapid in Group B and Group C compared to Group A till the end of experiment. There was an initial rise of alkaline phosphatase activity in wound tissue homogenate on 3rd day in all the groups. Thereafter, the level of alkaline phosphatase activity decline towards the end of the experiment, however the rate of reduction shown in was slow in Group A but rapid in Group B and Group C without much variation. The alanine transaminase activity of wound tissue homogenate was found higher on 3rd day in all the groups. Thereafter, the activity of alanine transaminase showed a declining trend toward the end period, which was recorded minimum in Group A compared to Group B and Group C. There was initial rise in the level of aspartate transaminase activity in wound tissue homogenate in all the groups on 3rd day observation, which gradually decreased toward the end of the experiment (9th day) in all the groups, however rate of decrease was slow in Group A compared to Group B and Group C. There was initial rise of creatine kinase activity in the wound tissue homogenate in all the groups on 3rd day observation. Thereafter, the creatine kinase activity in the wound tissue homogenate was declining towards the end of the experiment. The decreasing trend of creatine kinase activity in wound tissue homogenate was found rapid in Group B and Group C as compared to Group A.

The histopathological examination of the healing tissue taken on 9th day in Group B revealed thin layer of epidermal cells with a thin keratin layer without any scab. There was proliferation of fibroblast cells with collagen fibres deposition and formation of new capillaries directing towards the surface. In dermis hair follicles and sebaceous glands were observed (Fig.1). In Group C, histopathological examination revealed gradually detaching scab from the wound surface and formation of new epidermis with stratum granulosum under the scab. Under the stratum basale new capillaries containing few RBC were present. There was proliferation of fibroblast cell with collagen fibre deposition. The hair follicles and sebaceous glands were prominent (Fig.2). In Group A, some scab materials were still present on the wound surface on 9th day with a thin layer of squamous epithelial cells covering the wound surface. Focal area containing groups of hair follicles and sebaceous gland were seen in the dermis (Fig.3).

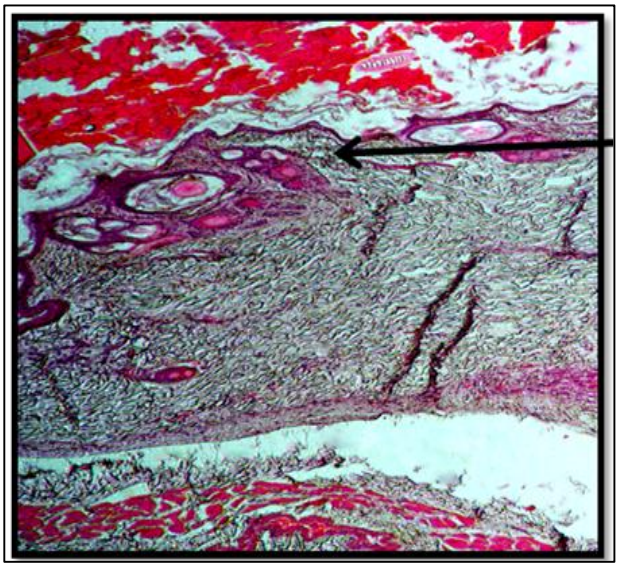

Fig 1: Section of wound tissue on 9th day showing scab with thin layer of epithelial cells with fibrous tissue proliferation in Group A. H&E× 10.

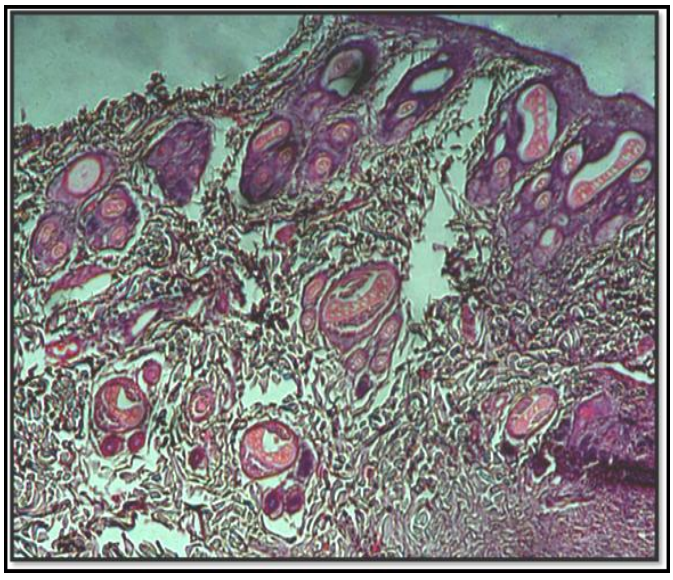

Fig 2: Section of wound tissue on 9th day showing thin keratin layer with fibrous tissue proliferation and angiogenesis in Group B. H&E× 10.

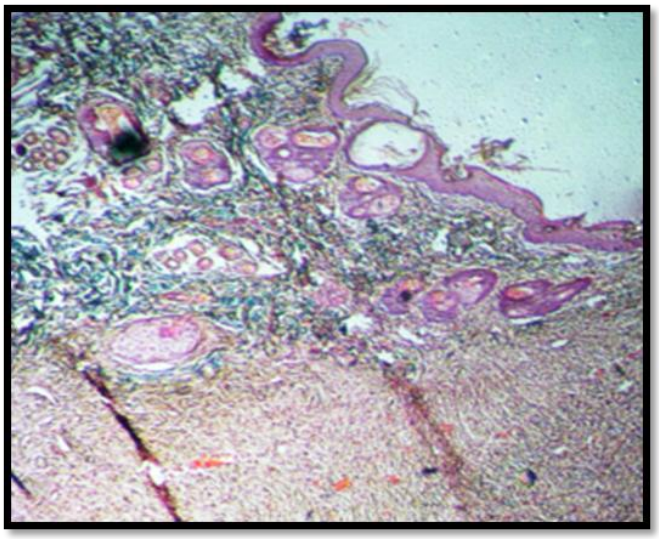

Fig 3: Section of wound tissue on 9th day showing detaching scab with fibrous tissue proliferation and angiogenesis in Group C H&E× 10.

Discussion

The maximum protein level of wound tissue homogenate was recorded on 9th day of experiment in all the groups. The rate of increasing trend in the protein level of wound tissue homogenate was observed faster in Group B and C while compared to Group A toward the end. It was observed that the variation in the activity of protein level in the wound tissues between the various treatment groups and between different days of treatment was highly significant (P<0.01). The interaction between the treatment groups and the days of treatment was also found to be highly significant (P<0.01).These observations were in accordance with the findings of Buffoni *et al.* (1993) [2] and Murthy *et al.* (2013) [12]. The increase in the protein level in wound tissue homogenate could be due to the synthesis of extracellular matrix protein by the macrophages during wound repair process. The collagen is the predominant extracellular protein in the granulation tissue of healing wound and synthesis increases soon after skin injury. Similar findings were also reported by Pandian *et al.* (2013) [14] and Murthy *et al.* (2013) [12].

There was an initial rise of alkaline phosphatase activity in wound tissue homogenate on 3rd day in all the groups. Thereafter, the level of alkaline phosphatase activity declined towards the end of the experiment; however, the rate of reduction was slow in Group A but rapid in Group B and Group C without much variation. Krotzsch *et al.* (2008) [9] also recorded similar initial rise in alkaline phosphatase activity in healing wound. The variation was highly significant (P<0.01) between the groups and between the treatment intervals. The findings were also supported by the findings of Dutta (2000) [5] in the pedicle omental grafted wound in bovine calves. The alkaline phosphatase activity in the site is an indicator for the healing status of the wound which gradually declined as the healing progressed. Similar findings were also reported by Rai *et al.* (2013) [15]. The increase in alkaline phosphatase activity of the wounded tissue could be attributed to the fact that healing process requires fibroblast for formation of granulation tissue. The findings were in close proximity with findings of Hawkins and Abrahamse (2007) [6], Dutta (2000) [5] and Iger and Abraham (1994) [8].

The Alanine transaminase activity of wound tissue homogenate was found higher on 3rd day in all the groups which shown a declining trend thereafter toward the end of the experiment. It was observed that the variation in the activity of alanine transaminase in the wound tissue homogenate between various treatment groups and between different days of treatment was highly significant (P< 0.01). The interaction between the treatment groups and the days of treatment was not significant. The initial rise of the alanine transaminase activity in the wound site probably due to tissue damage during inflicting the wound and the reduction of the same indicated the healing of wound. The findings were in accordance with the observations by Chiarelli *et al.* (1987) [3] in human burn patients and Hoopes and Ukr (1981) [7] in rats in cutaneous wound healing.

There was initial rise in the level of aspartate transaminase activity in wound tissue homogenate in all the groups on 3rd days of observation, which gradually decreased towards the end of the experiment (9th day) in all the groups, however, the level of aspartate transaminase activity was highest among the groups in each day of observation till the end of the experiment. The analysis of variance revealed n the activity of aspartate transaminase in the wound tissue homogenate between the various treatment groups and between different days of treatment was highly significant (P< 0.01). The interaction between the treatment groups and the days of treatment was not significant. The findings were in accordance with the observations by Chiarelli *et al.* (1987) [3] in human burn patients and Hoopes and Ukr (1981) [7] in rats cutaneous wound healing.

There was initial rise the creatine kinase level in the wound tissue homogenate in all the groups on 3rd day of observation, which declined as healing progressed. At the end of the experiment the creatine kinase activity in wound tissue homogenate was highest in Group A among the groups. The analysis of variance and the interaction between the treatment groups and the days indicates highly significant (P< 0.01) difference between groups and days of treatment. The interaction between the treatment groups and the days of treatment was also found to be highly significant (P<0.01). These observations were in accordance with the findings of Zheng *et al.* (1988) [17]. The rise of Creatine Kinase in the initial phase of wound tissue homogenate probably due to consumption of adenosine di-phosphate rapidly by damaged tissue and muscle which requires the enzyme Creatine kinase for its catalysis, however the activity declined as the catalytic phase in the wound site over owing to healing of the wound

toward the end. The findings were in accordance with the findings by Nordmann *et al*. (2009) [13] in humans.

The histopathological examination of the healing tissue taken on 9th day in Group B revealed thin layer of epidermal cell with a thin keratin layer without any scab. There was proliferation of fibroblast cells with collagen fibres deposition and formation of new capillaries directing towards the surface. In dermis hair follicles and sebaceous glands were observed. In Group C, histopathological examination revealed gradually detaching scab from the wound surface and formation of new epidermis with stratum granulosum under the scab. Under the stratum basale new capillaries containing few RBC were present. There was proliferation of fibroblast cell with collagen fibre deposition. The hair follicles and sebaceous gland were prominent. In Group A, some scab material was still present on the wound surface on 9th day with a thin layer of squamous epithelial covering the wound surface. Focal areas containing groups of hair follicles and sebaceous glands were seen in the dermis. Early fibrosis and Angiogenesis in Group B and Group C indicated faster healing as compared to Group A. The findings were in accordance with the observations of Mahmood *et al.* (2010) [11] and Hussein *et al*. (2011). According to Barua *et al*. (2013) [1] collagen provides strength and integrity to the dermis and all other supporting tissues, synthesis, secretion and subsequent organization of collagen play an integral role in wound healing.

Conclusion

Enzymatic and Biochemical findings of Wound tissue homogenate was suggestive of faster healing process in cutaneous wound in biomaterial treated animals. The histopathological findings of granulating wound samples were suggestive of accelerated healing process. The use of Bio-Ceramic Wound Treatment Device in chronic wound does not cause any tissue reaction and it is well accepted by the patient. From the current investigation, it was concluded that Bio-Ceramic Wound Treatment Device (Biomaterial) has a good healing property of wounds and may be considered for in-depth studies and further research programme.

References

1. Barua CC, Begum SA, Pathak DC, Bora RS. Wound healing activity of Alternanthera brasiliana Kuntze and its anti-oxidant profiles in experimentally induced diabetic rats. Journal of Applied Pharmaceutical Science, 2013; 3(10):161-166.
2. Buffoni F, Banchelli G, Cambi S, Vennelli G. Skin wound healing Some biochemical parameters in guineapig. J. Pharm. Pharmacol. 1993; 45(9):784-790.
3. Chiarelli A, Casadei A, Pornaro E, Siliprandi L, Mazzoleni F. Alanine and aspartate aminotransferase serum levels in burned patients: a long-term study. J. Trauma. 1987; 27(7):790-794.
4. Culling CF. Handbook of Histopathological and Histochemical Techniques. 3rd Edn. Butterworth and Co. (Published) Ltd. London, 1974, 446.
5. Dutta B. Omentoplasty for revitalization of ischaemic wound in bovines calves. Ph.D. Thesis, Assam Agricultural University, Guwahati, Assam, India, 2000.
6. Hawkins D, Abrahamse H. How Should an Increase in Alkaline Phosphatase Activity Be Interpreted? Laser Chemistry, 2007; 7:1-10.
7. Hoopes JE, Ukr BZh. Enzyme activities in regenerating epithelium during wound healing. IV. Aminotransferases and NADP-dependent enzymes. J. Surg Res. 1981; 15(4):262-70.
8. Iger Y, Abraham M. The process of wound healing in experimentally wounded carp. J. Fish Biol. 1994; 36:421-437.
9. Krotzsch K, Salgado RM, Caba D, Lichtinger A Padilla L, Di Silvio M. Alkaline Phosphatase Activity is Related to Acute Inflammation and Collagen Turnover During Acute and Chronic Wound Healing. Wound Repair and Regeneration, 2008; 13(2):28-48.
10. Luna LG. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed. McGraw-Hill, New York, 1968.
11. Mahmood AA, Mariod AA, Abdelwahab SI, Ismail SA. Potential activity of ethanolic extract of Boesenbergia rotunda rhizomes extract in accelerating wound healing in rats. J. Med. Plants Res. 2010; 4(15):1570-1576.
12. Murthy S, Gautam MK, Goel S, Purohit V, Sharma H, Goel RK. Evaluation of *In Vivo* Wound Healing Activity of Bacopa monniera on Different Wound Model in Rats. BioMed Research International. dx.doi.org/10.1155/2013/972028, 2013.
13. Nordmann G, Galbraith K, Mellor A. Raised creatine kinase as an indicator of inadequate muscle debridement in ballistic injuries. The Intensive Care Society, 2009.
14. Pandian C, Srinivasan A, Pelapolu IC. (2013). Evaluation of wound healing activity of hydroalcoholic extract of leaves of Stachytarpheta jamaicensis in streptozotocin induced diabetic rats. Der Pharmacia Lettre. 2013; 5 (2):193-200.
15. Rai AK, Saikia P, Mech B. Histochemical Localization ofAlkaline Phosphatase Activity during Cutaneous Wound Healing In a Catfish under Acid Stress. International Journal of Scientific and Research Publications. 2013; 3(8):50-53.
16. Snedecor GW, Cochran WG. Statistical methods. 8th Edn. Oxford and IBH Publishing Co, New Delhi, 1994.
17. Zheng LG, Wang ZG, Liu YQ, Chen XY, Ho SH. Reactions of serum creatine kinase in early phase of spherical steel bullet injury J Trauma. 1988; 28(1):225-227.