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Research Article

IN VIVO EVALUATION OF COMPOSITE WOUND DRESSING MATERIAL CONTAINING SOYA PROTEIN AND SAGO STARCH

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ABSTRACT

In this study we have reported the efficacy of the composite biomaterials made of soya protein and sago starch crosslinked with gluteraldehyde (SG-SY-G) as temporary wound-dressing materials using the rat as an animal model. Full-thickness excision wounds were made on the back of male rats weighing about 150 -200 g. The dressings were applied on the wounds and changed periodically at an interval of 4 days with the respective material. The wounds treated with SG-SY-G healed completely on 20th day after wound creation, whereas control showed complete healing only on the 25th day. Biochemical studies revealed a significant increase in total collagen, hexosamine and uronic acid contents in the granulation tissues of the treated wounds. The SG-SY-G composite acted an excellent wound dressing material, thereby absorbing excess exudates, and maintained a moist environment at the wound site. The enhanced wound healing in the experimental animals was reflected in the increased rate of wound contraction. The results of the histological and mechanical studies of the experimental groups revealed that reepithelialization and remodeling of the skin have been achieved by providing a moist environment at the wound site by the biomaterials and thereby hastening the migration of keratinocytes.

Keywords: Sago starch, Soya protein, Composite film, Wound healing.

INTRODUCTION

The skin provides primary protection against infection by acting as a physical barrier. When this barrier is damaged, pathogens have a direct route to infiltrate the body, potentially resulting in infection[1]. Healing is an intricate process initiated in response to an injury that restores the function and integrity of damaged tissue[2].In recent past considerable research has been done in the field of dermal substitution and wound healing. Nowadays, both artificial and natural polymers have been used to reconstitute dermis[3]. Healing process can be broadly categorized into inflammatory phase, proliferate phase and finally the remodeling phase which ultimately determines the strength and appearance of the healed tissue[4]. An ideal wound healing material should be non cytotoxic, maintain cell viability and should induce migration and proliferation of epithelial cells fibroblast and endothelial cells as well as extracellular matrix components required for wound repair[5]. The wound dressing material should exhibit ease of application and proper adherence, in order to ensure that no areas of non adherence left for bacterial proliferation[6]. An important objective in providing topical wound care is to select a wound dressing that should manage exudates and maintain a moist wound surface[7]. Natural polymers such as fibrin[8, 9], hyaluronic acid[10, 11], fibrinogen[12] and collagen[13-16] have been recently tested for local drug delivery and wound healing. The process of wound healing is promoted by several natural products[17], plant products, which are composed of active principles such as triterpenes and alkaloids [18] and biomolecules[19]. The wound healing potential of *Aloe vera*[20] and *Allium Cepa* Linn [21] was reported recently. Noorjahan *et al.*[22] prepared occlusive wound dressing materials based on physiologically clotted fibrin (PF) and its graft copolymers. Choudhary *et al* [23] studied the wound healing activity of ethanol extract of *Terminalia bellirica* Roxb fruit on exision and incision wound models. In recent years, collagen scaffolds are employed in tissue engineering such as skin, cartilage, bone and nerve as a support for cell mobility infiltration, proliferation and differentiation [24-26]. Senthil Kumar *et al* [27] used *triphala* extract incorporated in collagen sponge and its efficacy was evaluated in healing of infected dermal wounds. Gomathi *et al.*[28] used quercetin incorporated collagenous matrix for dermal wound healing in rat. Extracts of polysaccharide containing plants were widely employed for the treatment of skin and epithelium wounds[29]. Trombetta et *al*[30] reported about the importance of the extracts of polysaccharide containing plants that were widely employed for the treatment of skin and epithelial wounds and of mucus membrane irritation. The extracts of *Opuntia ficus-indica* cladodes were used in folk medicine for their antiulcer and wound-healing activities. The ideal dressing material need to ensure that wound remains moist and free of infection, while fulfilling prerequisites concerning structure and biocompatibility.

The film made of soya protein (SY) alone exhibits very poor mechanical properties, to improve these properties, sago starch (SG) was added to soya protein and SG-SY composite thus formed was cross-linked with glutaraldehyde (SG-SY-G). The SG-SY-G composites were used as wound dressing materials in experimental wounds of rats. The progress of the wound healing in both experimental and control groups was evaluated by macroscopic observations, planimetric studies, studies, histopathological and biochemical studies.

MATERIALS AND METHODS

Soya seeds and Sago rice were purchased from nearby local retail market and other chemicals used in this study were of laboratory grade.

Materials

Preparation and characterization of soya protein and sago starch film was already reported in our earlier studies[31].Briefly, the following stochiometric ratios are used for the preparation of SG-SY-G composite film. The ratios are 60 ml of 10% sago starch solution, 10 ml of 2% soya protein solution and 1µl of gluteraldehyde. The resultant solution mixture was poured in polythene trays having measurements 10×10 cm, dried at 30-35°C.The dried films were stored in polythene covers for further use.

Fabrication of SG-SY-G film for animal studies

The SG-SY-G films which gave better physico chemical properties were dried and sterilized by ethylene oxide (EtO) treatment and the films were soaked in gentamicin solution prior to the application on wounds.

Animal model

Seventy eight male albino Wister rats weighing 150-200 g were chosen, divided into two groups and the details are given in table 1. Throughout the experiment, rats were maintained in individual cages, under hygienic conditions and they were fed with commercial balanced diet with water *ad libitum*. The animal experiments were performed according to the institute's ethical committee approval and guidelines.

Table 1: Grouping of Animals.

Groups	Dressings	No. of Animals used for gross, biochemical and histological analysis						No. of animals used for the tensile strength analysis
		4th day	8th day	12th day	16th day	20th day	25th day	30th day
Group I	Control	6	6	6	6	6	6	6
GroupII	SG-SY-G	6	6	6	6	6	-	6

Surgical procedure and treatment

Each animal was given a dose of sodium pentobarbital 40 mg/kg body weight intraperitonally and the dorsal surface of the rat below the cervical region was shaved on its back under aseptic conditions. An open excision wound of 2×2 cm was created on the shaved dorsal side of rats using sterile surgical blade. For the control group, sterile cotton gauze dipped with gentamicin was applied on the wound. The test group was applied with the wound dressing film of SG-SY-G. The dressings were periodically changed at an interval of 4 days with the respective materials. Six rats were sacrificed periodically on 4th, 8th, 12th, 16th and 20th days of post wound creation and the granulation tissues formed were removed and stored at -70 °C until analysis. The progress of wound healing in both groups was evaluated by periodical monitoring of surface of the wound, histological and biomechanical studies.

Evaluation Studies

Photographic Evaluation

A visual proof of the healing pattern of the wound was provided by taking photos from a constant distance on 0, 4, 8, 12 16 and 20 days after wound creation.

Planimetric studies

Hair was clipped around the scar for proper visualization and the individual contour of the wounds of both control and experimental animals was measured, periodically, using a transparent graph sheet and the rate of healing was calculated and expressed as percentage contraction[32]

Histology studies

The animals were sacrificed periodically on 4th, 8th, 12th 16th and 20th days post wound creation and the tissue from the wound site of the individual animal was removed. These samples were then separately fixed in 10 % formalin, dehydrated through graded alcohol series, cleared in xylene, and embedded in paraffin wax (m.p. 56°C). Serial sections of 5 µm thickness were cut and stained with Hematoxylin and Eosin. The sections were examined under a microscope and photomicrographs were taken.

Biochemical parameters

Biochemical parameters at the wound site reveal the wound healing process. In the present study collagen, hexosamine and uronic acid levels were estimated in the granulation tissue of control and experimental wounds on days 4, 8, 12, 16, and 20.The granulation tissue was collected after sacrificing the animals on the respective days. Collagen and hexosamine were determined in defatted dried granulation tissue by the methods of Wossner[33] and Elson and Morgan[34], respectively. Extraction of uronic acid from the tissue was carried out according to the method of Schiller et al [35] and estimated by the method of Bitter and Muir[36].

Statistical Analysis

Data are expressed as means ± SD. Analysis of variance (ANOVA) followed by the student's t test was used to determine the significant differences among the groups. p Values less than 0.05 were considered significant[37]

RESULTS AND DISCUSSION

In recent past significant research has been carried out to improve the mechanical properties of the wound dressing materials. Better mechanical properties of wound dressing materials will be helpful for a surgeon to apply the same on to the contours of the wound. If the material is brittle or its mechanical properties decreases when it comes in contact with water or wound exudates, the material cannot be applied properly on to the wound surface.

Animal studies

Period of Epithelialization

The macroscopic analysis of the wound revealed that the SG-SY-G dressing treated group took only 20 days for complete epithelialization, whereas the control groups took 25 days for complete epithelialization. It was observed that the healed area of SG-SY-G treated animals was regular and uniform compared to that of control groups.

Wound contraction studies

The details of wound contraction in both control and experimental groups are given in figure1. On day 4, the wound contraction of control was 10 % whereas that of the experimental was 18 %. However, by day 16, 73 % of the wound was closed in the experimental groups. Almost complete closure of the wound was observed on 20th day in the experimental animals, whereas only 85 % of the wound was closed in the control animals. This shows the efficacy of the SG-SY-G as a wound dressing material on the wound surface.

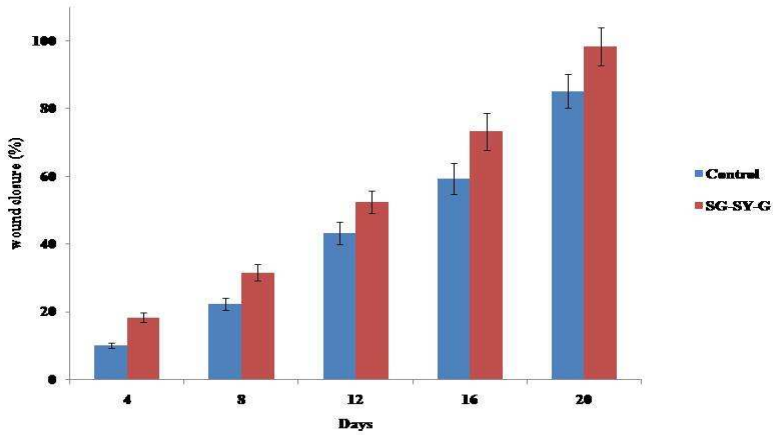

Fig. 1: Higher percentage of wound closure was observed in the wounds treated with SG-SY-G compared to control.

Photographic Evaluations

Surface of the wounds were photographed periodically from a constant distance for both control and experimental. Faster rate of healing was observed in the experimental wounds compared to those of control. The results are in agreement with those of planimetric observations.

Biochemical Parameters

The biochemical parameters i.e. collagen, hexosamine and uronic acid in the granulation tissues of the control and experimental rats on different days after wound creation was analyzed. The collagen contents of both control and experimental increased up to day 12 and later decreased (Fig.2). However the collagen contents have shown higher values on all the days studied in experimental animals. The marked increase in collagen content of granulation tissue isolated from experimental groups may be due to increased synthesis of collagen and could be correlated with the effective healing of wounds[38, 39].

The hexosamine values have shown decreasing trend in both control and experimental groups (Fig. 4). However there is a notable increase in the experimental groups compared to control group. Similar trend was observed in the contents of uronic acid (Fig. 5). Increase in the levels of the three biochemical parameters i.e. collagen, uronic acid and hexosamine in the experimental groups gave an indication of the faster rate of wound healing compared to controls. Similar results have observed in the earlier studies[22, 27, 40].

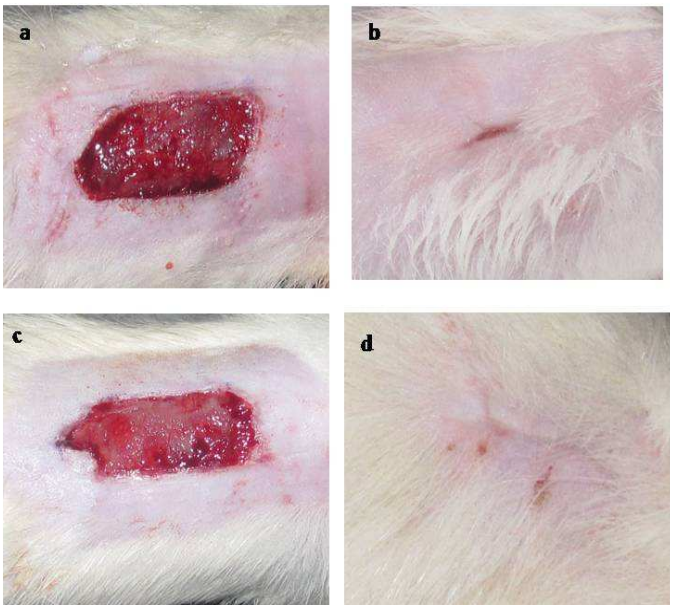

Fig. 2: Photographic evaluation of wounds; Faster rate of wound healing was observed in the SG-SY-G treated wounds on 20th day (d). However, incomplete wound closure was observed in the control wounds (b).

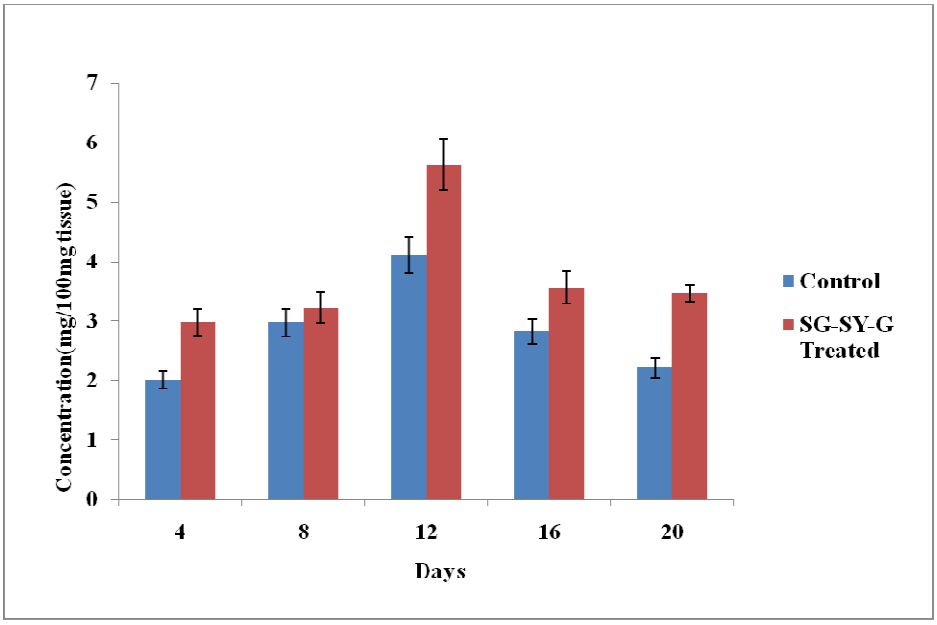

Fig. 3: The collagen content of granulation tissue in control and SG-SY-G treated wounds. The collagen contents of both control and experimental rats increased up to 12th day and later decreased; similar trend was observed control animals also. However, collagen content showed higher values on all days studied in experimental animals.

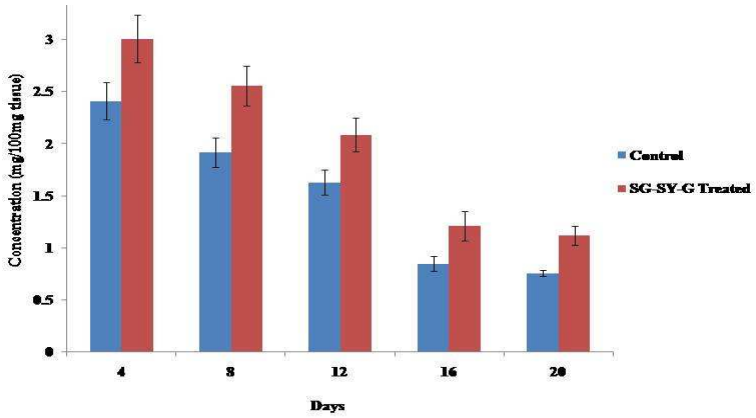

Fig. 4: Hexosamine values of the granulation tissue of control and experimental animals. Hexosamine values have shown decreasing trend in both control and experimental groups. However, higher values were observed compared to those of control groups.

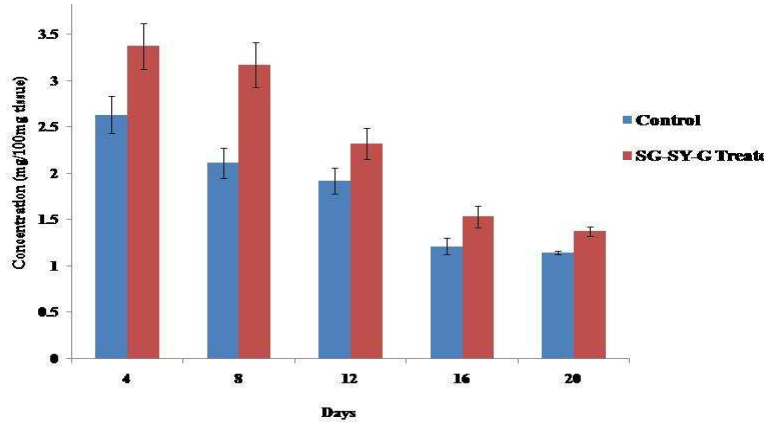

Fig. 5: Uronic acid values of the granulation tissue of control and experimental animals. Uronic acid values have shown decreasing trend in both control and experimental groups. However, higher values were observed compared to those of control groups.

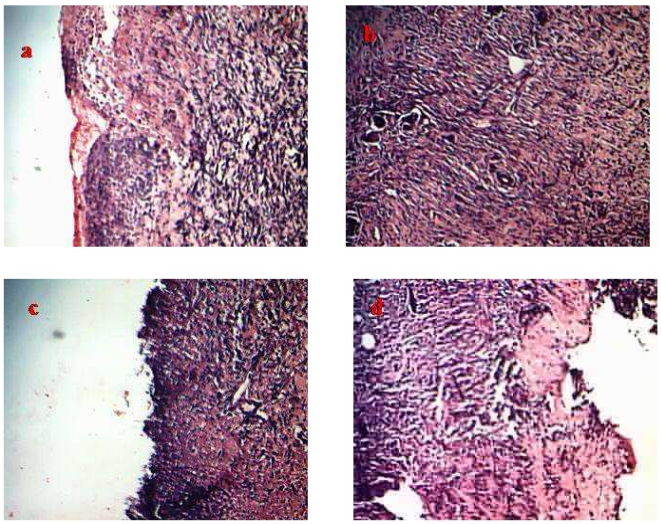

Fig. 6: Histological studies of the control and experimental wounds; (a) on day 4, control wound has shown skin with ulceration along with inflammatory cell response. (b) On 20th day histological section of control wound showed regenerated epidermis with fibrocollagenous tissue beneath. (c) On day 4, histological section of experimental wounds exhibited inflammatory cell response. (d) On 20th day histological section, experimental wounds has shown normal histological pattern of healthy skin.

Histological Studies

Histological section of the control wound on day 4 has shown skin with ulceration and more inflammatory cell response. On 12th day in the control wound no epithelial lining was observed over the ulcer. Inflammatory granulation tissue was seen beneath the ulcer along with vertically arranged collagen bundles.

On 20th day regenerating epidermis was observed with fibrocollagenous tissue beneath it. On 25th day normal histological

response was observed i.e. generally seen in healthy skin. In the case of experimental wounds treated with SG-SY-G composite, on 4th day inflammatory cell response was observed. On 12th day regenerating activity is seen in the epidermis with dense inflammation beneath the ulcer, more collagenous tissue was observed. On 16th day healed wound with complete covering by regenerating epidermis along with mature collagen beneath it was observed. No adnexal structure was observed. On 20th day normal histological pattern of healthy skin was observed. The histopathological studies reveal that wound healing was complete by 20th day in the case of SG-SY-G treated wounds whereas normal histology was observed in the control wounds only on 25th day.

Tensile Strength of healed skin

Wound healing, a fundamental response to tissue injury, is a complex process involving a series of biological events, and occurs by a process of connective tissue repair. A fibrous scar is the end product of this process, the predominant constituent of which is collagen. There is a rapid increase in the synthesis of this protein in the wound area soon after an injury. In addition to providing strength to a tissue matrix, collagen also plays an important role on homeostasis. Biochemical components such as amino acids and fatty acids are important for the synthesis of collagen fibers and recovery is hindered if these are deficient. Table 3 gives the breaking strength of excision wounds of control and experimental groups. The tensile strength exhibited by experimental groups is greater than that exhibited by control group. Increased tensile strength indicates increase in collagen matrix. There is rapid biosynthetic activity in experimental groups during initial phase of granulation. In the remodeling phase, maturation of collagen takes place by the formation of inter and intramolecular cross links; hence, the wound strength is increased. But in the case of control, the decreased tensile strength and prolonged wound healing further support the slow rate of wound healing due to dry wound-healing conditions offered by cotton gauze.

Table 3: Tensile strength of healed skin of control and experimental

Wound dressings	Elongation At Break (%)	Tensile Strength (MPa)
Control-Gauze	141.11 ± 10.75	0.37
SG-SY-G	96.94±2.58	0.52

Values are expressed as mean ± SD for six animals in each individual experiment. Within a line, values without a common letter are significantly different from the control group at $p < 0.05$ as determined by ANOVA.

Among the experimental groups, those treated with SG-SY-G show higher tensile strength values when compared with control, and this is mainly attributed to its increased hydrophilic capacity, which is reflected in its faster wound-healing nature. Moreover, the tensile strength is directly related to the amount of collagen synthesized at the wound site. If we observe the results shown in Fig. 10D**,** the collagen content in the SG-SY-G treated wounds was higher when compared to control group at all intervals.

CONCLUSION

Evaluation of the SG-SY-G composite as a temporary biological wound dressing material on the experimental wounds of rats has revealed that the experimental wounds healed faster than the control wounds. The gross observations have revealed that the complete closing of wounds were observed by the 20th day for the animals treated with SG-SY-G, whereas it took 25 days for control wounds. These observations gave an indication that SG-SY-G composite might be tried as a wound dressing material in the clinical wounds of smaller and larger animals before apply on to humans.

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