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Research Article

DEVELOPMENT AND CHARACTERIZATION OF BAMBOO BASED WOUND DRESSING COATED WITH NATURAL EXTRACTS OF CURCUMIN, *ALOE VERA* AND CHITOSAN ENHANCED WITH RECOMBINANT HUMAN EPIDERMAL GROWTH FACTOR AND *IN VIVO* EVALUATION FOR WISTAR ALBINO WOUNDED RATS

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ABSTRACT

Development of natural fibres based wound dressing material coated with different compositions of aloe vera, curcumin and Chitosan enhanced with rhEGF for wound healing applications was explicate in this research work. The coated fabrics were subjected to mechanical properties, anti-bacterial test and Scanning Electron Microscopy. Justifying more on these findings, the coated fabric samples were tested for its wound healing efficacy was studied by in vivo method of wound healing using Wister Albino rats. The dorsal surface of the rat's skin was removed and created a full – thickness wound. Then the developed fabric samples loaded with rhEGF were applied over the wound surface and the wound closure was studied in equal interval of days. The formation of zone of inhibition in antibacterial test and wound closure rate in In Vivo evaluation reveals that a specified composition of natural extracts of Aloe vera, curcumin and solution of Chitosan enhanced with rhEGF reveals that coated fabric samples exhibits good antibacterial property and higher rate of wound healing nature. Hence therefore these coated fabrics can be suitable for faster wound healing process.

Keywords: Wound dressing, Aloe vera, Curcumin, Chitosan, rhEGf, In Vivo,

INTRODUCTION

Wound is the result of disruption of normal anatomical structure and function[1,2] and also wounds can be classified according to their tissue loss i.e., wounds without tissue loss (eg, in surgery) and wounds with tissue loss (which includes burn wounds, wounds caused by trauma and diabetic ulcers). Normally wound dressings have been used to cover wounds and absorb bleeding[3].

Wound-healing process consists of four highly integrated and overlapping phases: hemostasis, inflammation, proliferation, and tissue remodeling or resolution[4,5]. There are many factors that can affect wound healing which interfere with one or more phases in this process, thus causing improper or impaired tissue repair[4].

In olden days the role of wound dressings were very minimum in healing of wounds [6,7]. An ideal wound dressing should protect the wound from bacterial infection, provide a moist and healing environment, and be biocompatible[8].

The emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy[9]. In this regard, researchers are increasingly turning their attention to herbal products[10]. For thousands of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs. The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief. It is estimated that local communities have used about 10% of all flowering plants on Earth to treat various infections, although only 1% have gained recognition by modern scientists[11].

Textiles with antimicrobial properties are gaining interest. There is a great demand for textiles with antimicrobial or self-cleaning properties. With the growing concern for the environment, the use of natural products to impart various functions to textiles has attracted increasing attention[12].

Due to the potential toxicity to humans, and environmental concerns about the use of some of these biocides, naturally-occurring and biocompatible compounds are preferable for use in wound dressings and other medical textile applications.

Curcumin is a herbal compound found in turmeric (Curcuma longa L.). The WHO (World Health Organization) and FAO (Food and Agriculture Organization) have verified its safety as a natural food colorant (C.I. Natural Yellow 3)[13,14].

Aloe vera has been used for many centuries for its curative and therapeutic properties and although over 75 active ingredients

from the inner gel have been identified, therapeutic effects have not been correlated well with each individual component[15,16].

Chitosan–curcumin formulations are already being successfully used as part of a biological sponge, which is used as a medical device for wounds and swellings during dislocation healing. The chitosan was responsible for the swelling reduction and wound healing, at the same time as the curcumin influenced the increasing growth of collagen and other connecting tissues[17,18].

The greatest benefit of an ideal antimicrobial treatment of textiles will be obtained only when it satisfies a number of requirements. The efficacy of the treated textile material should be very high against a broad spectrum of bacterial and fungal species with low toxicity, allergy or irritation to the end users. The surface properties of the product should not be changed after laundering, dry cleaning and hot pressing. The physical properties and the appearance of the textile should not change after finishing. The finishing process should be cost effective and it should not release any harmful substance to the producer and the environment[19].
In the present study a wound dressing material coated with natural extracts is developed and coated with rhEGF, and investigated for its drug-releasing efficacy on wounds. To gain further knowledge about the developed wound dressing material with respect to wound healing, in vivo study was also undertaken.

MATERIALS AND METHODS

The Bamboo yarn (100 %) of Ne 40s was purchased from M/s. Cheran spinning mills, Salem, Tamilnadu, India. Procured yarns were weaved as fabric in M/s. Sun Power Bit Looms, Pallipalayam, Tamilnadu, India. Curcumin powder (95% purity) was purchased from Biotan Pharma, Chennai, Tamil Nadu. Aloe Vera leaves in raw form were collected from Erode. The rhEGF as REGEN-D™ was purchased from M/s. Bio Plus, Coimbatore. Deionized water was used for all experiments.

Curcumin extract Preparation: An exact amount of curcumin powder was suspended in absolute ethanol in order to prepare a 0.5wt% solution. The suspension was continuously stirred for 12 hours to ensure complete dissolving[17].

Aloe Vera extracts Preparation: Collected leaves of the Aloe vera plant were completely washed, shadow dried and powdered. Double the volume of powder taken was mixed with absolute ethyl alcohol. Continuous stirring of the mixture was done and it was left ideally for 24 hours, after which it was filtered and same volume of ethyl alcohol was mixed as used earlier. The same procedure was repeated two times. Produced three filtrates were mixed, and the content of alcohol was evaporated naturally at room temperature which leads to obtain a concentrated aloe extract (0.5%). The powdered Aloe vera extract was redissolved in double distilled water[20].

Chitosan solution preparation: Chitosan solution was prepared by measuring an exact amount of chitosan powder and it was suspended in ethanol in order to prepare 0.5 wt % solution with continuous stirring of solution for 1 hour at 60º C. Pure distilled water was used whole through the process[17].

Coating on fabric: The coating process was carried out on a laboratory-scaled padder (Werner Mathis AG, Switzerland). The pressure between the pads was set at 2 bars and the rotation speed to 2 m/min. A wet pick-up of 80% was achieved using these settings. The impregnated fabrics were dried in a hot air dryer (Werner Mathis AG, Switzerland) at 80ºC for 15 minutes[17]. The fabrics were subjected to standard atmosphere conditions for 24 hours after the drying process. (Table 1).

Incorporation of rhEGF on the coated fabric
The predetermined EGF (Table 1) was dissolved in 100 ml of distilled water and constant stirring was carried out for 10 mins. Then the developed fabrics were coated with rhEGF as described in Table 1.

Measurements of Bamboo fabric properties: The woven bamboo fabric properties like ends per inch and picks per inch were counted using the counting glass. The tearing strength, tensile strength, abrasion resistance, fabric thickness and fabric stiffness were determined as per the D5587-15, D5035-11, D4157-13, ASTM D1777-96 and ASTM D6828 standard methods respectively. The areal density was measured using GSM cutter method as per ASTM D3775.

Antibacterial test: Antibacterial efficacy was assessed using AATCC 147 standards. The coated fabric samples were tested for antibacterial activity against Gram-negative (*Escherichia coli*) and a Gram-positive bacteria (*Staphylococcus aureus*). The line of incubation of antimicrobial agent was shown by the presence of growth inhibition zones measured by using Muller-Hinton (HiMedia) [21,22].

Scanning Electron Microscopy: Surface Morphology and cross – section of the coated samples were studied by scanning electron microscope (SEM, JEOL, JSEM-6390LV, Japan).

Animal test Methods
Ethical Issue

All animal procedures were according to guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals and Institutional Animal Ethics Committee (IAEC) approved by M/s. Nanda College of Pharmacy, vide proposal No. NCP/IAEC/No: 8/2014 -15.

Wound Creation: Male Wistar Albino rats were used for the study. Animals weighing about 180 ± 50 g were selected for the animal models. All animal procedures were according to guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals and Institutional Animal Ethics Committee (IAEC) approved by M/s. Nanda College of Pharmacy, vide proposal No. NCP/IAEC/No: 8/2014 -15. Total of 30 rats were taken for study and they were divided into 6 groups. Hair – removal cream was used to shave the skin of the animal and cleaned with ethanol (96 % concentration). A full – thickness wound was created and size of the wound was approximately 2.5 cm long and 2 cm wide. Wounds were created in group 1 and no treatment was done on it. For group 2 rats wounds were created and treated with the commercially available drug Povidone-Iodine (PVP-I) for every two days. In group 3, wounds were created and treated with control group (Curcumin, Aloevera, Chitosan) for every 5 days. In the remaining 3 groups wounds were created and treated every 5 days with the developed wound dressing samples. The wound healing rate was observed by measuring wound closures on 0^{th}, 2^{nd} , 5^{th}, 7^{th}, 10^{th}, 12^{th}, 15^{th}, 17^{th}, and 21^{st} day after wound creation[23].

Wound Closure Observation: The photography of wound closure was captured using Nikon digital camera (Model: Nikon Coolpix S6700 Point & Shoot Camera) at the time interval mentioned earlier. The percentage of wound closure was

calculated as follows by the initial and final area using graph paper during observation. The wound size reduction was calculated as follows:

$$\% \text{ of Wound size reduction} = D_i - D_f / D_i \times 100$$

Where D_i is the initial area of wound on 0^{th} day and D_f is the area of wound at the time of treatment with coated fabric on 2^{nd}, 5^{th}, 7^{th}, 10^{th}, 12^{th}, 15^{th}, 17^{th}, and 21^{st} day accordingly.

RESULTS

Mechanical Properties of Coated Bamboo Fabric: The mechanical properties of bamboo fabric were measured before and after coating and the results are shown in Table 2.

Antibacterial Test: The developed fabrics were subjected to test antimicrobial efficacy against *Escherichia coli* and *Staphylococcus aureus* through agar diffusion test method. The samples were grouped in the way that group 1 was untreated fabric, group 2 was fabric treated with the commercially available drug Povidone-Iodine (PVP-I) (Control Group) and group 3, 4, 5 and 6 (already named as CAC, CAC I, CAC II and CAC III respectively) were fabrics coated with the ratio mentioned in Table 1.

Morphological study of the coated fabrics: The morphology of the coated fabric samples were studied using SEM. The images shown in figure 2. The extent of penetration of extract coated on the fabric reveals that the different extracts prepared coated were penetrated interior into the fabric which plays a major role in wound healing property of the developed wound dressing material.

In Vivo Evaluation: Figure 3 shows the wound healing rate of samples measured in duration as mentioned earlier.

Table 1: Preparation ratio of Curcumin, Aloe Vera, Chitosan and rhEGF

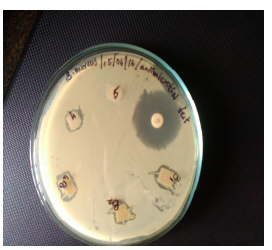
Group A	100 % Bamboo Yarn			
Sample	CAC	CAC I	CAC II	CAC III
Curcumin (ml)	20	20	30	40
Aloevera (ml)	20	20	20	20
Chitosan (ml)	30	30	30	30
rhEGF (µg)	-	5	10	15

Table 2: Mechanical Properties of Bamboo fabric

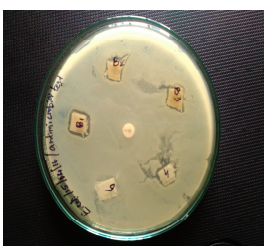
S No	Properties	Before Coating	After Coating			
			CAC	CAC I	CAC II	CAC III
1	Weave	Plain				
2	Ends/inch(EPI)	76				
3	Picks/inch(PPI)	64				
4	Areal Density	39.4 g/m²	39.5 g/m²	39.5 g/m²	39.5 g/m²	39.6 g/m²
7	Thickness mm)	3.8 mm	3.8 mm	3.8 mm	3.8 mm	3.8 mm
8	Tearing strength Warp - Weft -	 1600 g 2432 g	 1624 g 2468.4 g	 1625 g 2470.5 g	 1624 g 2469.4 g	 1600 g 2470.2 g
9	Tensile strength Warp - Weft -	 78lbs 69lbs	 79.1 lbs 70.0 lbs	 79.3 lbs 71.0 lbs	 79.2 lbs 70.1 lbs	 79.2 lbs 71.0 lbs
10	Abrasion resistance	5.8 %	5.9 %	5.8 %	5.9 %	5.9 %

Table 3: Results of Antibacterial Test

S.No	Samples	Name of Organisms (Zone of Inhibition in diameter (mm))	
		Staphylococcus aureus	*Escherichia coli*
1	Untreated fabric	0	0
2	Control group	20	18
3	CAC	11	10
4	CAC I	12	10
5	CAC II	21	19
6	CAC III	28	24



(a)



(b)

Figure 1: (a) Formation of zone of inhibition against *Staphylococcus aureus* and (b) Formation of zone of inhibition against *Escherichia coli*

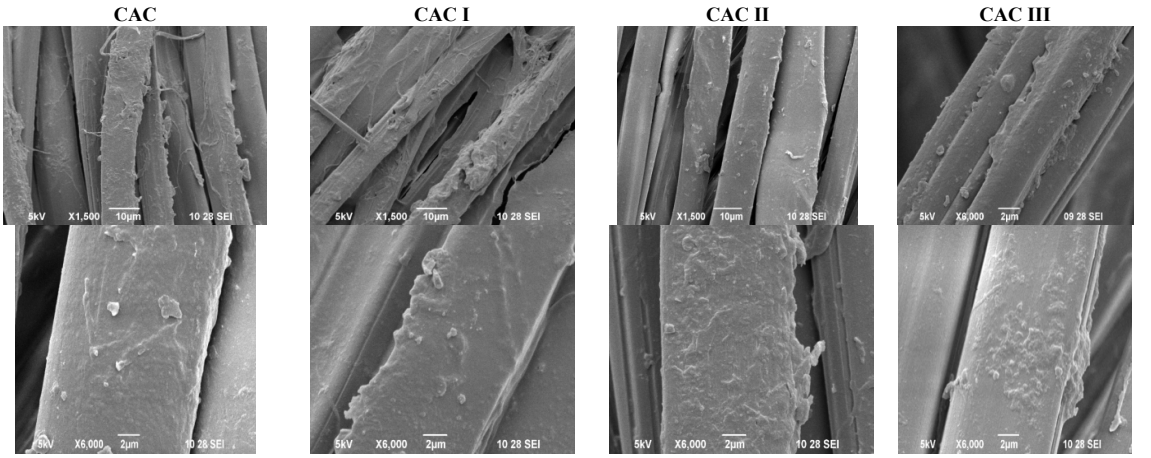


Figure 2: SEM micrographs of the coated Bamboo fabrics

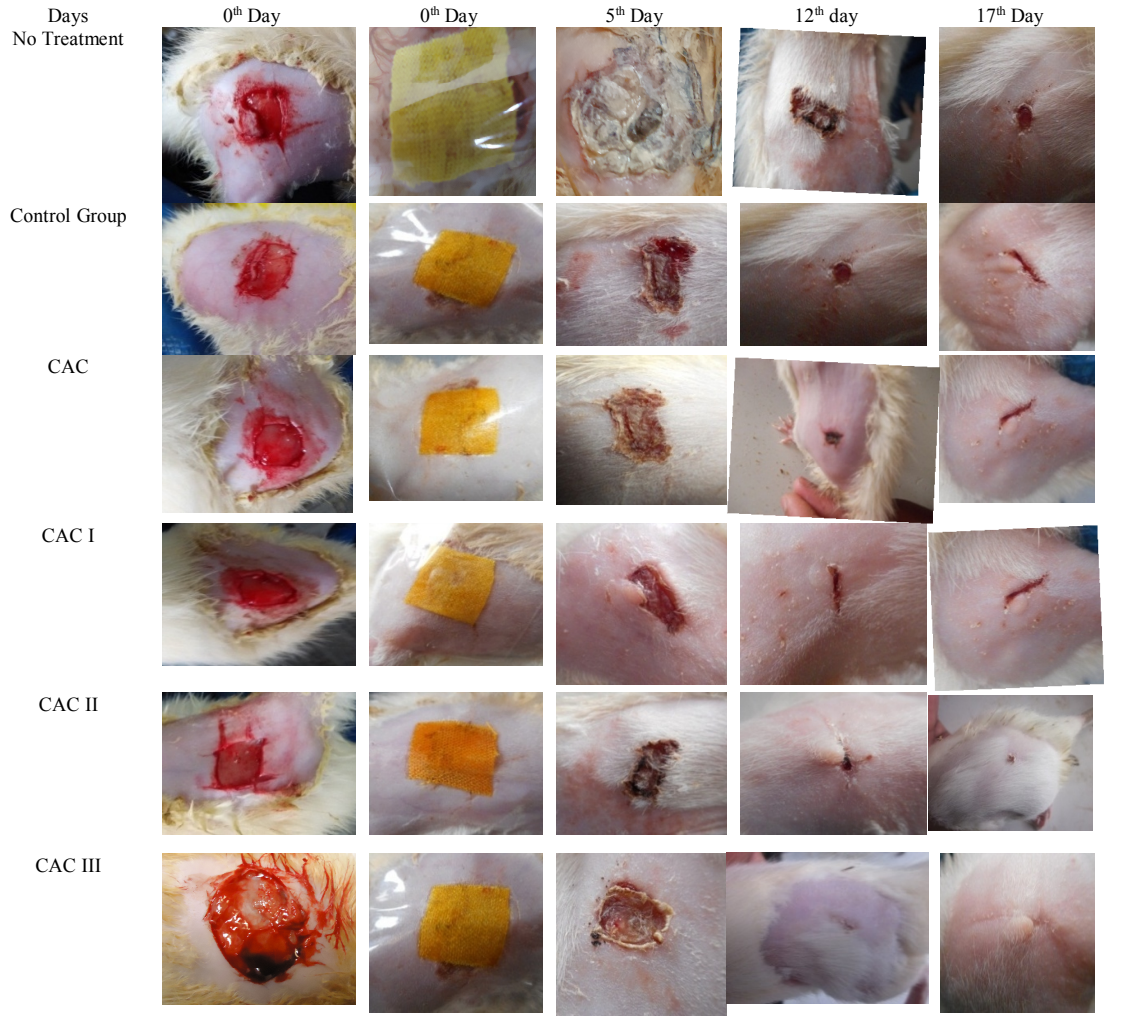


Figure 3: Wound Closure Study

DISCUSSION

The results of mechanical properties of the coated fabric shows that the areal density of the fabric before coating is 39.47 g/m² and it increase with 1.5 % after coating. Similarly the thickness, tearing strength, tensile strength and abrasion resistance increases at a considerable level after coating.

The comparative analysis of the test results of antibacterial activity is shown in the Figure 1. The zone of inhibition is high against *Escherichia coli* and *Staphylococcus aureus* in the sample CAC III which is about 24 mm and 28 mm after 24 hrs of inhibition. From all the samples CAC III shows better antibacterial property which is evident from the zone of inhibition as shown in figure 1. This also concludes that as the ratio of curcumin & rhEGF is increased the better will be the antibacterial property.

The morphological study of coated fabric as shown in figure 2 reveals that extracts coated binds evenly over the surface of the fabric samples. The images clearly shows that porous nature of the fabrics were not affected after coating with Aloe vera, Curcumin extracts and Chitosan solution.

The results of In vivo evaluation were compared and shown in figure 3 which reveals that sample 6 has faster wound closure rate. Figure 3 shows the images of wound size reduction after treatment with coated wound dressing on 0^{th}, 5^{th}, 12^{th} and 17^{th} days. The size reduction of wounds shows faster wound healing rate when compared with other compositions (Wounds not treated, treated with commercially available drug Povidone-Iodine (PVP-I) and other ratios of Aloe vera extracts, Curcumin extracts and Chitosan solution enhanced with rhEGF)

CONCLUSION

In the present study the efficacy of coated bamboo fabric used as a wound dressing material in which the extracts of Aloe vera, Curcumin and solution of Chitosan are coated and enhanced with rhEGF is investigated. Antibacterial test result shows excellent result with good formation of zone of inhibition in the CAC III. Similarly in vivo evaluation results reveals the CAC III (20 ml Aloe vera extract, 40 ml of Curcumin extract and 30 ml of Chitosan solution enhanced with 15µg of rhEGF) has good antibacterial activity as compared with other samples. It is further proven that natural extracts with Chitosan solution coated bamboo fabrics enhanced with rhEGF can be used as a wound dressing material with wound healing properties.

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