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In Vivo Wound Healing Study of Chitosan Turmeric Films in Rat Model

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ABSTRACT

Hydrogel based wound dressings are flexible, permeable to oxygen and conformable with more comfort over traditional dressings. In the present study bioactive wound dressing material composed of chitosan with turmeric extracts, in the form of film was prepared. Physical and mechanical properties of the films, enhancing rate of wound healing were examined. The elongation percent for chitosan film + methanol turmeric extract, chitosan film + turmeric oil and chitosan film (control) was found to be 80.05%, 73.22% and 43.97%, respectively. Chitosan films with turmeric extracts showed good elongation. Water absorption capacity for chitosan-methanol turmeric, chitosan-turmeric oil and chitosan film was found to be 0.757g/g, 0.515g/g and 0.410g/g, respectively. Water vapor permeability of the films was also calculated. Permeability for chitosan-methanol turmeric, chitosan-turmeric oil and chitosan film was found to be 1.463g/cm^2, 1.593g/cm^2 and 1.31g/cm^2, respectively. Wound healing efficacy was evaluated for chitosan-methanol turmeric, chitosan-turmeric oil and chitosan films using rat model. Wounds treated with chitosan-methanol turmeric film were healed in 15 days with complete epithelization. Period of epithelization for chitosan-turmeric oil film treated wound was 18 days. Control film treated and untreated wounds healed in 21 days. Percent wound contraction decreased rapidly for chitosan-methanol turmeric film treated group compared to untreated group. Wound healing promoting features of chitosan and curcumin in turmeric made chitosan-based films a potential wound dressing material.

KEY WORDS: Hydrogel, Chitosan, Turmeric, Wound healing.

1. INTRODUCTION

The skin acts as a natural barrier protecting the body against physical and chemical damage. Physical injuries resulting in break or opening of skin or loss of cellular and anatomic continuity of living tissue is turned as a wound (Demidova, 2012; Pastar, 2014). Delay or failure in wound healing forms chronic wounds, under unfavorable conditions increase in tissue destruction and necrosis are likely to occur (Demidova, 2012; Anderson and Hamm, 2012; Eming, 2014). Initiation of wound healing process is essential to prevent microbial infection and occurrence of chronic wounds. The dynamic process of wound healing involves inflammation necessary for hemostasis and clot formation, fibroplasia, formation of granulation tissue, re-epithelialization and tissue remodeling (Qing, 2017; Singh, 2017; Peng-Hui Wang, 2018). Basic function of any wound care product is to provide moist warm environment, protection against infection and absorption of exudates (Anderson and Hamm, 2012; Pop and Almquist, 2017; Vowden and Vowden, 2017; Koehler, 2018). Besides this, dressing material is expected to possess properties like ease of application and removal, bio-compatibility and cost-effectiveness.

Turmeric is extensively used as a spice and food preservation in Eastern cuisine. In ayurvedic medicine, turmeric was used as a household remedy to treat certain disorders and wounds. Extensive study on turmeric has proved its wound healing capacity. Curcumin (diferuloylmethane), main bioactive component of turmeric exhibits a wide spectrum of biological actions such as anti-inflammatory, anti-oxidant, anti-thrombotic, antimicrobial, and anti-carcinogenic activity (Gong, 2013; Kant, 2014, Fahimi, 2015). Safety evaluation studies indicated that very high dosage of turmeric and curcumin are well tolerated without toxic any effects, thus making them a potential for development of wound dressing material in modern medicine.

Chitosan is a derivative of chitin, a polysaccharide, found abundantly in crustacean exoskeletons of crab, shrimps and lobsters. This naturally occurring polysaccharide, which can form hydrogels are widely exploited for its use in tissue engineering, drug delivery and cell encapsulation (Kang, 2008; Park, 2009; Ma, 2010; Archana, 2016; Rajitha, 2016). Chitosan has been prepared as a biomaterial with a wide range of biomedical and industrial applications because of its biological activitiessuch as biodegradability, biocompatibility, antimicrobial activity, and stimulation of healing. Chitosan is positively charged and is soluble in aqueous solution of acids forming a viscous homogenate. In modern medicine, chitosan attracts researchers due to its film forming characteristics with unique functional and biomedical properties. Chitosan is capable of promoting cellular proliferation and differentiation, which could also be enhanced by incorporating growth inducing factors (Muzzarelli, 2011). Chitosan can accelerate wound healing activity at every stage of healing by enhancing the functions of inflammatory cells (Ueno, 2001; Santos, 2007) macrophages (Kojima, 2004) and fibroblasts (Howling, 2001; Nascimento, 2009; Wiegand, 2010).

In the present study, chitosan films were prepared by incorporating turmeric extracts. Three different films namely chitosan turmeric, chitosan turmeric oil and chitosan films were formulated, for which physical and mechanical properties, water absorption capacity and water vapor permeability were studied. Further, wound healing

efficacy of the different films was tested using rat model. The main aim of the study is to prepare a potential wound dressing material with chitosan and turmeric.

2. MATERIALS AND METHODS

Preparation of Turmeric extracts: Fresh rhizomes of *Curcuma longa* were collected from local market. The rhizomes (200g) were ground to a fine paste with sterile water using a mechanical blender. Turmeric oil was extracted from the rhizome paste by steam distillation. The residue was filtered using Whatman filter paper no.1 and air dried. Residue was suspended in 200ml chloroform in a sterile Erlenmeyer flask overnight and the contents were filtered. Residue was suspended for re-extraction in 200 ml methanol overnight. The mixture was then filtered and the filtrate was collected in a wide Petri dish. Methanol was completely evaporated under vacuum condition to obtain the oily residue and was dissolved in 10 ml DMSO and stored at 4°C.

Preparation of Films: Chitosan (2%, w/v) was added to acetic acid (1%, v/v) and lactic acid (1%, v/v) in a sterile 50ml beaker and stirred for an hour using magnetic stirrer to obtain homogenous viscous solution. 1.5ml of turmeric extracts (methanol turmeric extract or turmeric oil) was added to the homogenate and stirred again. The homogenate was spread evenly on glass moulds to obtain films with uniform thickness and was air dried at room temperature for 48 hours in sterile conditions. The films were gently peeled off using 0.5 mM NaOH and rinsed with sterile water to remove excess NaOH. The films were stored at room temperature in air tight bags.

Study of Physical and Mechanical properties of the Film:

Thickness: Film thickness was determined using thickness meter and for each film measurements were taken at 10 different locations. The values are represented as the average of three measurements.

Mechanical Properties: The elongation of the films was determined using a texture analyzer – Instron equipped with a 5 kg load cell. A thin film strip (dimensions 8cm x 2.5cm) was held in between two clamps and pulled by a top clamp at a rate of 30mm/min. The load at break (N) and elongation % (mm) are measured when the film broke off. The values are the average from 3 measurements. Tensile strength and elongation of the film at break were calculated by the following equations:

$$\text{Tensile Strength (N/mm}^2\text{)} = \frac{\text{Breaking force}}{\text{Cross sectional break of the sample}}$$

$$\text{Elongation at break (\%)} = \frac{\text{Increase in length at breaking point(mm)}}{\text{Initial length(mm)}} \text{ x } 100$$

Water Absorption Capacity: The films of similar weight (weighed initially) were immersed in 50ml phosphate buffered saline (PBS, pH 7.4) and incubated at room temperature. At a consistent time interval of 30 minutes, films were taken out, removed excess water by blotting them on a filter paper and weighed until it reaches a constant value (Remunan and Bodmeier, 1997). Water absorption capacity of the films was calculated using the formula:

$$\text{Percent water absorption capacity} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \text{ x } 100$$

Water Vapor Permeability: Films were tied to the mouth of borosilicate glass bottle (capacity 15ml;diameter of top hole,16mm) filled with anhydrous calcium chloride. All the bottles were weighed initially.On an average, area of 8.042 cm^2 is available for permeation of vapor. Bottles were placed in desiccators containing saturated sodium chloride solution (35.9g/100ml) maintained at room temperature. Open bottles were considered as control (Chung, 2003). After 24 days, final weight of the bottles was measured in triplicate using a weighing balance.

***In vivo* wound healing study:** The *in vivo* wound healing characteristics of the prepared films were evaluated using rat model. Twelve adult wistar rats weighing approximately 200-250g approved by Institutional Animal Ethics Committee, PSG Institute of Medical Sciences and Research (proposal ref:124), Coimbatore (Tamil Nadu), were used in this study. The rats were housed under standard conditions (12 h light and 12 h dark cycle at 25±30°C) with free access to food and water. Rats were anaesthetized using diethyl etherprior to excision to wound. Single layer wound of area 1.7cm^2 was excised using sharp scissors and forceps on dorsal side by removing circular piece of skin. Animals were divided into 4 groups: Group 1 was left untreated, Group 2 was treated with chitosan film, Group 3 chitosan film + turmeric oil and Group 4 chitosan film+methanol turmeric extract. The wounds were covered completely by films, followed by placing gauze on the top. The films were secured in place by using an adhesive tape. Wounds were traced on 1mm^2 graph paper once in every three days until complete epithelization, to measure the wound area. The period of epithelization was expressed as the number of days required for eschar falling with no visible raw wound (Nayak, 2007). Wound contraction percentage was calculated using the following formula:

$$\text{\% wound contraction} = \frac{\text{Initial wound area} - \text{Specific wound area}}{\text{Initial wound area}} \text{x } 100$$

Statistical analysis: Statistical analysis was carried out by one way analysis of variance (ANOVA) to compare the wound area contraction. Values of $^*p<0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

Chitosan films with turmeric extracts were prepared as mentioned in the methods section. Elasticity of the dressing is one of the primary properties for an ideal dressing material.On measuring the thickness of films, it was known that thinnest film with maximum elongation property could be obtained by adding methanol turmeric extract to the chitosan homogenate. Mechanical strength of the films was in the range of 2.21 N to 6.59N and elasticity values ranged between 43.97% and 80.05%. In agreement with the earlier report (Dhurai, 2013), tensile strength of the film changed in inverse proportion to its elasticity. The mechanical and physical properties of the films are tabulated in table.1.

Table.1. Mechanical and Physical properties of films

Film name	Tensile strength (N/mm^2 ± SD)	Film elongation (% ± SD)	Mean thickness of the film (mm ± SD)	Water vapor permeability (weight gained in g/cm^2 of the film) (g ±SD)	Water absorption capacity (%)
Chitosan	0.017 ±0.001	43.97 ±13.18	158±20.43	1.31 ±0.034	41
Chitosan-turmeric oil	0.351 ±0.002	73.22 ±11.72	139±60.45	1.59 3±0.683	51
Chitosan-methanol turmeric	0.286 ±0.009	80.05 ±5.32	124±22.70	1.463 ±0.240	75

Previous literature stated that it is essential to keep the moisture in control during healing process with balanced absorption of fluid from wound bed (Kant, 2014; Fahimi, 2015; Karri, 2015). Water absorption capacity of different films varied between 0.4g/g and 0.757g/g, showing an increased absorption capacity with respect to increase in time interval. Chitosan methanol turmeric film was found to have higher water absorption capacity. Since oxygen and moisture plays an important role in wound healing process, film dressings with optimum water vapor permeability is more advantageous in treating wounds (Gong, 2013; Rajitha, 2016; Archana, 2016; Pop and Almquist, 2017; Koehler, 2018). Water vapor permeability of the films lied between 1.31g and 1.593 g. Chitosan film incorporated with turmeric oil seemed to have high water vapor permeabilitycompared to other two films. Chitosan-methanol turmeric films showed optimal water vapor permeability, promising its sustainability for better wound healing activity.

Animal study was carried out with 12 wistar rats in 4 groups. The rate of wound healing seems to be similar for group treated with chitosan film and untreated group. It was reported that, no significant difference was observed for wounds treated with chitosan acetic acid and lactic acid films compared to untreated wounds. Wound treated with chitosan methanol turmeric and chitosan-turmeric oil film healed within 15 and 18 days, respectively. Whereas, chitosan film and untreated group took 21 days for healing. Complete epithelization was observed between days 12 and 15, 15 and 18 for chitosan methanol turmeric and chitosan-turmeric oil films, respectively.

Rapid and accelerated wound contraction was seen in group treated using chitosan methanol turmeric film with 98.7±0.43% contraction on day 15. Group treated with chitosan-turmeric oil film also exhibited significant percent of wound contraction, 95.60±0.29% on day 15. Percent wound contraction for chitosan film and untreated group was 82.0±0.41% and 83.7±0.32%, respectively. The above results were in accordance with Dhurai, 2013.

It is clear from figure.1 and table.2, that wounds treated with film containing turmeric extracts healed significantly in a short period of time compared to control. The study indicates that chitosan turmeric and chitosan-turmeric oil films are effective and beneficial for wound healing.

Table.2. Effect of different films on percentage of wound contraction

Days (D)	Untreated	Chitosan	Chitosan-Turmeric Oil	Chitosan-methanol Turmeric
0	0.00	0.00	0.00	0.00
3	23.32 ± 0.83	13.26 ± 0.26	46.01 ± 0.25	64.48 ± 0.44
6	35.73 ± 0.28	26.32 ± 0.20	63.94 ± 0.09	74.72 ± 0.15
9	45.87 ± 0.39	36.61 ± 0.37	71.98 ± 0.41	80.72 ± 0.47
12	55.08 ± 0.46	44.80 ± 0.37	78.80 ± 0.55	88.48 ± 0.42
15	83.71 ± 0.32	82.07 ± 0.41	95.60 ± 0.29	98.67 ± 0.43
18	92.40 ± 0.52	94.63 ± 0.51	99.22 ± 0.38	100.00
21	98.82 ± 0.40	98.24 ± 0.00	100.00	100.00

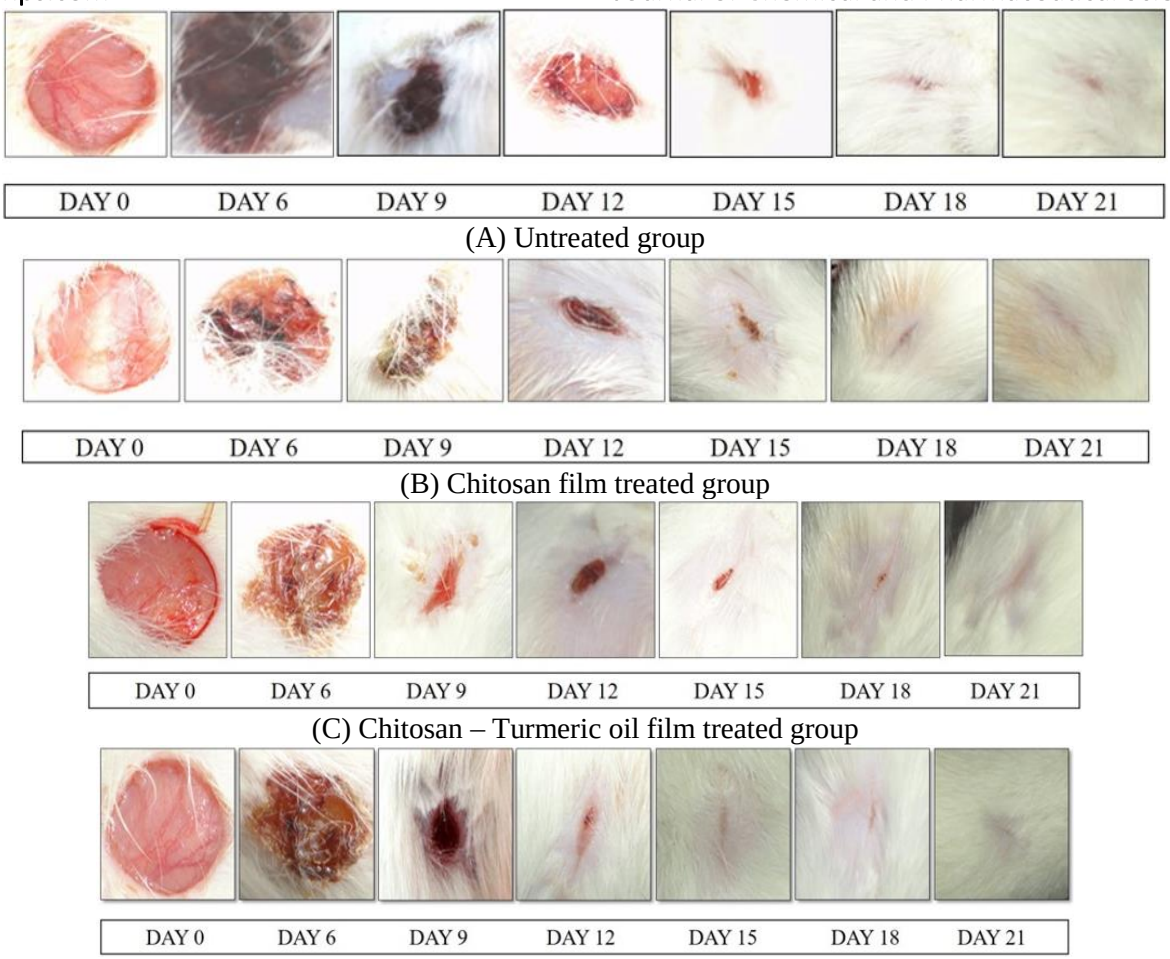

Figure.1. Photographic representation of % wound contraction for excision wound on rats

4. CONCLUSION

Chitosan films incorporated with turmeric extracts were prepared. Thickness, mechanical and physical properties of the different films was examined. Chitosan methanol turmeric film seemed to have good elongation property compared to chitosan-turmeric oil film and chitosan film. Furthermore, water absorption capacity and water vapor permeability were calculated for the films.Results showed optimal water vapor permeability for chitosan methanol turmeric film with high rate of water absorption capacity.Wound healing efficacy for various films was tested on rat model. Wound contraction area was measured and calculated percent wound contraction, since the initiation of wound until complete epithelization. Wounds treated with chitosan–methanol turmeric film and chitosan-turmeric oil film healed faster within 15 and 18 days, respectively. Wounds treated with chitosan film and untreated wounds took 21 days to heal. From the above results it is understood that chitosan film with turmeric extracts can be developed into a potential wound dressing material, capable of accelerating wound healing activity by providing moist environment.

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