

A preliminary investigation of Turmeric-Agar composite film as bioactive wound dressing material on excision wound in rat model

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Curcuma longa Linn. is used in herbal medicines as antiseptic for wound healing. The objective of the present study is to investigate the turmeric impregnated agar film as bioactive wound dressing material. The turmeric-agar composite film was prepared by mixing, the acetone or methanol extract of turmeric powder and agar added with or without gelatin, glycerol, propylene glycol as additives. Physical and mechanical properties of the film were tested for its suitability for wound coverage. The antibacterial activity test of the film showed significant reduction in cell density as compared to the control. Turmeric-agar composite film applied wound showed complete epithelization by 11 days for turmeric-agar with acetone extract whereas 10 days for turmeric-agar with methanol extract when compared to 14 days in the control.

Keywords: Antibacterial, *Curcuma longa*, Turmeric, Turmeric-Agar film, Wound dressing material.

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Introduction

Damaged skin is a good medium for entry and growth of microbes; hence, management of wound is the immediate step to protect the body against infection. A wound may be defined as a break in the epithelial integrity of the skin or may also be defined as a loss or breaking of cellular and anatomic or functional continuity of living tissue¹. Wound healing is a natural phenomenon in which damaged epidermal tissue is regenerated by ordered wound healing process. Wound healing is enhanced by many factors. Wound healing studies are mainly aimed to detect various factors influencing healing process, so they could be either used or avoided in clinical practice to favourably alter the healing process². Turmeric is used widely as a spice in Indian cuisine and as medicine. Turmeric powder was the earliest substance used for wound healing. There are many reports showing turmeric has wound healing activity^{3,4}. Curcumin is a group of alkaloids that exerts its wound healing activity. In combination with honey, fresh paste of turmeric was used on full thickness wounds⁵. Turmeric extract showed bacteriocidal activity against

many of the Gram positive and Gram negative bacteria except few like *Campylobacter jejuni*⁶.

An effective wound dressing should not only protect the wound surface from the surrounding but also should have other desirable properties like accelerating wound healing, biocompatibility, biodegradability and cost effectiveness. Modification of wound atmosphere is another way of promoting wound healing. The wound dressing should cover the wound and able to provide correct microenvironment, which accelerates wound healing process. Wound dressing made out of gelatin and epidermal growth factor exhibited wound healing property⁷. The film forming wound dressing material has added advantages as it can be applied directly on the wound. Agar is a natural polysaccharide available in plenty. The film prepared from agar alone does not have considerable tensile strength and elongation⁸. Extensive work has been done on *C. longa* and demonstrated its antimicrobial, antioxidant and wound healing properties. It is being used in creams and cosmetics. In this paper, we have made an attempt to prepare an agar based composite film by incorporating turmeric extract. We also explored the potential of developing a bioactive wound dressing material with turmeric extract in the form of film.

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Materials and Methods

Plant material preparation

Dried rhizomes of *C. longa* collected from local market were ground into fine powder using mechanical blender. The fine powder was obtained after sieving using 150 µm sieve. The fine turmeric powder (10 g) was resuspended in 100 ml of organic solvent (methanol or acetone) in a sterile Erlenmeyer flask with intermittent shaking for 72 hours. The mixture was filtered through Whatman filter paper No.1 and the filtrate was collected in wide glass plate. The solvent was completely evaporated under vacuum and the oily residue thus obtained was dissolved in DMSO.

Film preparation

Agar-agar, plasticizer (glycerol or propylene glycol) and gelatin were added to distilled water and boiled for 2-4 min to obtain a homogenous solution. Methanol or acetone extract of turmeric (4% v/v) was added to the solution and poured into a glass mould. The cast was then air dried at room temperature for 48 h to obtain film as shown in Plate 1. The film was carefully removed by peeling away from the mould and stored in air tight containers for further use.

Study of physical and mechanical properties of film

Mechanical properties

The film thickness was measured at ten different locations using thickness meter. The mechanical properties were measured using a texture analyser (Instron equipped with a 5 kg load cell). A film strip (8 cm × 2.5 cm) was held between two clamps and pulled at a rate of 30 mm/min⁹. The load at break (N), extension at break (mm) and time at break (sec) were measured when the film tore off.

Water absorption

The water absorption was studied by soaking the pre-cut films (of similar initial weight) in 50 ml

of phosphate-buffered saline (PBS, pH 7.4) for defined period at 37°C. Every 30 min time intervals, the excess water was blotted out and the weight of the film was measured until it reached a constant value¹⁰. Water absorption capacity was calculated using the following formula:

Per cent water absorption capacity =

$$\frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Water vapour permeability

The film was tied to the mouth of the glass bottle (capacity, 15 ml, diameter, 16 mm) filled with predetermined anhydrous calcium chloride. The average area available for vapour permeation is 8.042 cm². All bottles were placed in a desiccator containing saturated sodium chloride solution (35.9g/100 ml) maintained at room temperature¹⁴. After 24 days, the final weight was measured and the percent weight gain was calculated. Bottle without film was taken as control¹¹. The water vapour permeability of the film was measured using the following formula:

Rate of moisture permeability (mg/day/l) =

$$\frac{1000 \{ (T_f - T_i) - (C_f - C_i) \}}{10V}$$

Where;

T_f-Final weight of the bottle with film,

T_i-Initial weight of the bottle with film

C_f-Final weight of the bottle without film,

C_i-Initial weight of the bottle without film

V: Volume of the bottle

Antimicrobial activity

Turmeric-agar composite film was cut into squares (1.3 cm × 1.3 cm), and immersed in 20 ml sterile Luria broth, in 25 ml conical flasks¹². The medium

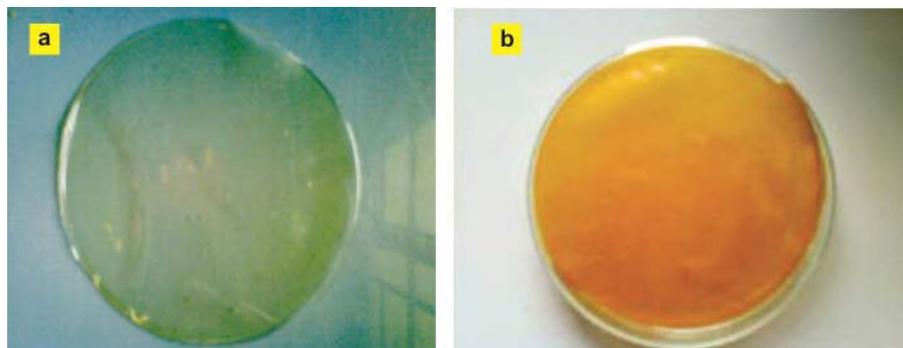


Plate 1 — Agar composite film: A- without turmeric extract, B- with turmeric extract

was inoculated with 1% overnight grown culture (log phase) inoculum of the test bacteria and flasks were placed on an orbital shaker maintained at 37°C at 200 rpm. Agar composite film without the turmeric extract was taken as negative control. Agar composite film with silver nitrate (4%) was taken as the positive control. Two ml culture was sampled periodically at different time intervals (0, 2, 4, 8 and 24 h) and cell density at 600 nm was measured using UV-visible spectrophotometer (ELICO SL 159). To calculate the reduction in cell density, control culture without film was taken as 100% density for each bacterial strain.

Animal study

The animal study was approved by the Institutional Animal Ethics Committee, PSG College of Pharmacy, Coimbatore. Healthy male rats weighing 240-300 g were used for the present study. They were housed and maintained on normal food and water. The animals were anaesthetized using ketamine (80mg/kg body), prior to and during infliction of the experimental wounds. The particular skin area was shaved and two impressions were made on the dorsal thoracic region 1 cm either side, away from the vertebral column and 5 cm away from each ear on the anaesthetized rat. The skin of impressed area was excised to full thickness to obtain a wound area of about 204 mm². Twelve animals were divided into three groups, Group 1: (Control), Group 2: AGGP-TME, (Agar-Gelatin-Glycerol-Propyleneglycol-Turmeric Methanol Extract) Group 3: AGGP-TAE (Agar-Gelatin-Glycerol-Propyleneglycol-Turmeric Acetone Extract). After wound creation, control and one of the test films were applied to the two wounds of the same rat to eliminate inter-individual differences. Wound area was measured by tracing the wound on a millimeter scale graph paper on

predetermined days i.e., 0, 4, 8, 12 and 14 days post-wounding. Percent of wound contraction was calculated taking the initial size of the wound (204 mm²) as 100% using following formula:

$$\% \text{ Wound contraction} = \frac{\text{Initial wound area} - \text{Specific day wound area}}{\text{Initial wound area}}$$

Falling of the scab or hair growth leaving no raw wound was taken as the point of complete epithelization and the days required taken as period of Epithelization¹³.

Statistical analysis

The means of wound area contraction measurements, between the test and control were compared using one way ANOVA. The results were expressed as mean \pm SEM.

Result and Discussion

Agar composite films were prepared in various combinations (Table 1). The film prepared with agar alone was sticky and the agar with gelatin film was non-sticky but very stiff. Film with glycerol or propylene glycol was non-sticky and soft. Various glycol derivatives such propylene glycol and polyethylene glycol can also be used to plasticize polymeric films. Plasticizers function by weakening the intermolecular attractions between the polymeric chains and generally reduce the tensile strength and increase the flexibility¹⁴. AGGP films were flexible and transparent. As the film will be subjected to stress during practical usage, determination of mechanical properties such as tensile strength and elongation is important. When turmeric extract was added to the AGGP film, elongation, thickness and water absorption was increased, whereas tensile strength was reduced (Table 2). Antimicrobial test was done

Table 1—Codes, composition and nature of agar based films prepared

Composition of the film	Agar conc. (%)	Gelatin conc. (%)	Glycerol conc. (%)	PG conc. (%)	Nature of the film
Agar	1.5	-	-	-	Sticky
Agar-Gelatin	1.5	1.5	-	-	Very Stiff
Agar-Gelatin-Glycerol	1.5	0.5	2.0	-	Translucent, non-sticky
Agar-Gelatin-propylene glycol (AGP)	1.5	1.5	-	0.5	Transparent, non-sticky
Agar-Gelatin-Glycerol-Propylene glycol (AGGP)	1.5	0.5	1.5	0.5	Transparent, non-sticky

Each experiment was repeated minimum of three times.

Table 2— Mechanical and physical properties of turmeric-agar films

Code of the Film	Tensile strength (N/mm ² \pm SD)	Film elongation (% \pm SD)	Mean thickness of the film (mm \pm SD)	Water vapour permeability (weight gained in g/ cm ² of film) (g \pm SD)	Water absorption (% \pm SD)
AGGP	0.051 \pm 0.002	13.8 \pm 0.0	177 \pm 5.70	2.2 \pm 0.13	96
AGGP-TME	0.007 \pm 0.001	20.4 \pm 0.0	289 \pm 2.16	1.8 \pm 0.18	184
AGGP-TAE	0.036 \pm 0.003	25.5 \pm 4.5	394 \pm 10.70	2.4 \pm 0.30	237

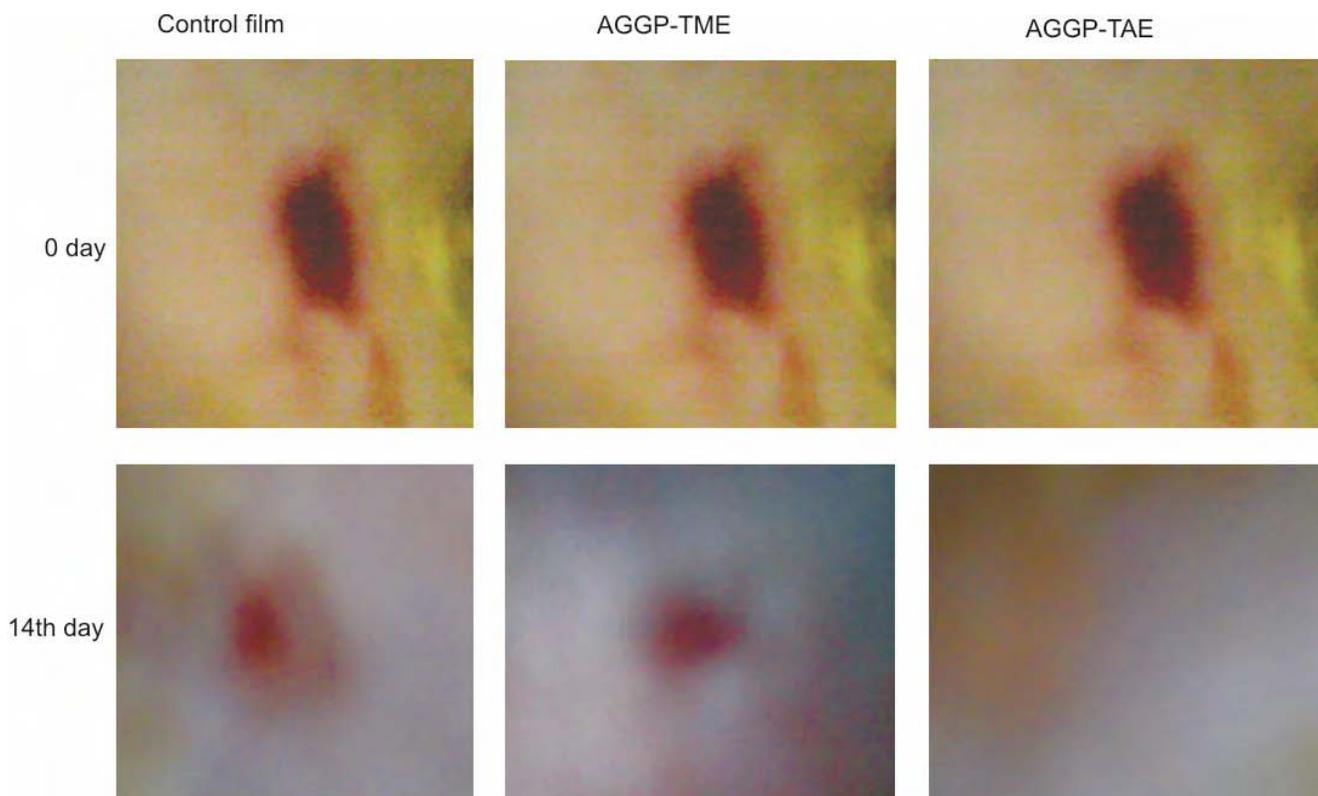


Plate 2 — Effect of turmeric extract impregnated films on excision wound model

Table 3—Antimicrobial testing of turmeric-agar film against different bacterial strains

Code of the Film	Per cent reduction of cell density (OD _{600nm})			
	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Proteus vulgaris</i>	<i>Klebsella</i> sp.
Control Broth	-	-3	-	-
AGGP-TME film	24	29	34	18
AGGP-TAE film	25	26	32	13
AGGP Film with AgNO ₃	27	41	25	27

Each experiment was repeated minimum of three times.

against four different organisms as shown in Table 3. Turmeric extract incorporated film showed reduction in cell density compared to control films but it was lesser than the positive control film (agar film with silver nitrate). It may be attributed to low release of the turmeric extract into the culture broth. Similar study was reported for starch based film¹⁵. Wound contraction progresses faster in case of treated wound when compared with the control (Plate 2). The

complete healing of wound was observed between 10th and 14th days. AGGP-TME film showed 93.6±0.5% degree of contraction whereas AGGP-TAE film showed 98.8±0.3% degree of contraction and the control film showed only 86.1±0.7% degree of contraction on the 10th day (Table 4). In terms of period of epithelization also, the AGGP-TAE film showed growth of hair on 10th day but it was not observed completely until 14th day in the control. The accelerated wound contraction and epithelization period may be due to the growth promoting nature of *C. longa* extract.

Conclusion

The results of the present investigation clearly indicated that the turmeric incorporated agar composite film accelerated the wound healing process. Further, turmeric based bioactive wound dressing material as a film has a great potential to heal the wounds. Therefore, there is an ample scope for the transparent sheet prepared from agar composite film incorporated with turmeric bioactive compounds to apply for real life situations such as burn wounds and surgical wounds.

Table 4—Effect of turmeric –Agar composite film on excision wound models in rat

Code of the Film	Degree of contraction (%)						
	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14
Control film	8.33±3.04*	27.77±2.77*	48.61±3.34*	77.77±1.75*	86.11±0.70*	93.41±0.79*	99.44±0.35*
AGGP-TME	22.22±4.12*	38.88±3.51*	65.27±2.56*	80.55±1.75*	93.60±0.51*	99.17±0.37*	100*
AGGP-TAE	24.99±4.30*	44.44±2.77*	70.83±1.86*	83.33±0.0*	98.88±0.35*	100*	100*
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
F value	5.345	7.778	18.909	3.750	140.76	46.9	-

*Values are mean ± SEM

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