

AN WOUND DRESSING MATERIAL USING CHITOSAN IMPREGNATED WITH EMBELIN

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ABSTRACT

The prevalence of wounds in India is 4.5 out of 1000 persons most of which being lower extremity wounds. When left untreated the infections may spread throughout the body and can lead to severe complications like sepsis. Wound healing is the process in which the skin repairs itself after an injury. Chitosan and Embelin are natural compounds which possess high wound healing activity. Chitosan is extracted from the shells of *Perna indica* by the deacetylation of chitin and Embelin is extracted from berries of *Embelia ribes* by maceration process. The wound dressing material is made from the predetermined concentration of chitosan-embelin mixture. The material is characterised using XRD to determine its structure, FTIR to determine its organic and polymeric materials, antibacterial activity and MTT assay in fibroblast cell lines.

KEYWORDS: *Sepsis, Embelin, Chitosan, maceration, wound healing, fibroblast cell lines.*

1. INTRODUCTION

Wound healing is the disrupted state of the tissues caused by the physical, chemical, microbial or immunological activities ultimately leading to healing of the injured site. The prevalence of wounds in India is 4.5 per 1000 with the lower extremity wounds being the most common. The wound healing process basically involves four phases: hemostasis, inflammation, fibroblast proliferation, remodelling. Wound dressings are used to avoid all shortcomings which occurred during wound healing process. Modern dressings include hydrocolloids, alginates, hydro gels, semi permeable adhesive film, foams, biological dressings and tissue engineered skin substitutes. The wastes from the food industry as shrimp shells, mussel shells, have the natural polymers which is the major source of surface pollution in coastal areas. Every year some 6 million tonnes to 8 million tonnes of waste crab, shrimp, and lobster shells are produced globally. Among which about 1.5 million tonnes is produced in south East Asia alone. This chitinous solid waste fraction of average Indian landing of shell fish was ranged from 60,000-80,000 tonnes. In developing countries, waste shells are being dumped in

landfills and near the sea shores. In developed countries like Australia the disposal of such wastes is costly as much as US \$150 per tonne. Chitosan acts as a healing agent that helps in inhibiting the fibroplasia formation which promotes the tissue growth and development.

2. MATERIALS AND METHODS

2.1 Sample Collection

The chemicals used were purchased from Marck Specialities private limited which are of analytical grade. The sample *Perna indica* was collected from the market of Valliathura of Thiruvananthapuram district in Kerala and the berries of *Embelia ribes* that are commonly available.

2.2 Extraction of Chitosan

The chemical method is being followed in the isolation of chitosan. This involves various steps as: Deproteinisation, Demineralisation and Deacetylation. The deproteinisation of mussel shells was carried out by soaking the shells in 2M NaOH at an ambient temperature ($27\pm 2^\circ\text{C}$) for about 48 hours. The residual shells were washed with distilled water until a neutral pH is attained. The demineralisation process is done by soaking the shells in 1M HCl at ambient temperature for the next 24 hours after which the shells were again washed with distilled water to obtain neutral pH. The obtained result is called chitin. The deacetylated chitin is called as chitosan, for the removal of acetyl groups from chitin the shells were soaked in 2M NaOH for 48 hours. This process is called as deacetylation. The shells obtained were neutralised with distilled water and were dried and powdered.



Fig 1: Chitosan powder

2.3 Extraction of Embelin

The samples collected were dried and powdered. To obtain embelin, Maceration process is administered in which 1:10 ratio of sample is soaked in ethanol and is left undisturbed for 7 days. The

filtrate is kept in hot air oven at 60°C for vapourisation. The crude extract obtained is then stored at -20°C.



Fig 2: Embelin extract

2.4 Preparation of wound dressing material using Chitosan

Chitosan film was made by mixing chitosan with a linking agent and a few drops of ethylene glycol. Under slight heating and stirring the mixture is dissolved. After cooling the mixture is poured into the paraffin made plates and the set up is left undisturbed for about 3 days. After 3 days a flexible, tear resistant chitosan film remains, which is easily peeled off the paraffin plate.



Fig 3: Chitosan Film

2.5 Preparation of wound dressing material using Chitosan-Embelin

Chitosan-embelin film was made by mixing chitosan with embelin and a few drops of ethylene glycol and a linking agent. Under slight heating and stirring the mixture is dissolved completely. After cooling the mixture is poured into the paraffin made plates and the set up is left undisturbed for about 3 days. After 3 days flexible, tear resistant chitosan-embelin film remains, this is easily peeled off the paraffin plate.



Fig 4: Chitosan-Embelin Film

3.RESULTS AND DISCUSSIONS

3.1 Antibacterial test

The materials of chitosan, and chitosan-embelin mixture were tested for antibacterial activity in the gram positive bacteria *Bacillus subtilis*. The activity was evaluated which showed that chitosan-embelin mixture had more effective activity when compared to that of chitosan. The zone was measured to be of 1cm in diameter.

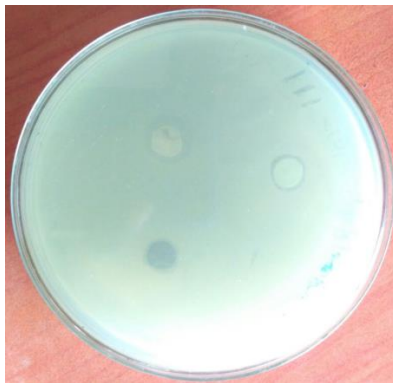


Fig 5: Antibacterial test

3.2 X-ray diffraction

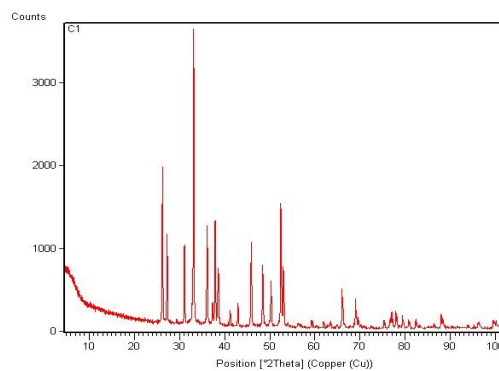


Fig 6: XRD spectrum of Chitosan

The X-ray diffraction pattern of the obtained chitosan exhibits strong reflections at 2θ value of $9-12^\circ$ and mirror reflections at higher degree of 2θ . The peak intensities analysed from the graph are 26.998, 28.037, 32.778, 37.401, 43.917 and 53.928 at 2θ . The crystalline reflection of chitosan sample is observed in the range of $30-40^\circ$ at 2θ .

3.3 Fourier Transform Infrared Spectroscopy

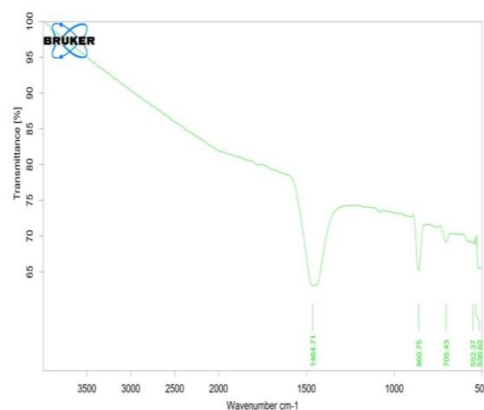


Fig 7: FTIR spectrum of Chitosan

The peaks at 1464.71cm^{-1} showed the OH and CH deformation ring of chitosan. The peak at 860.75cm^{-1} describes the NH wag primary amine in chitosan.

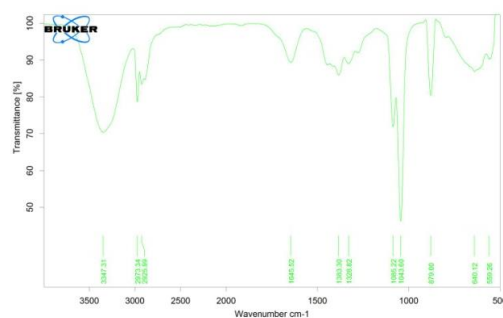


Fig 8: FTIR spectrum of Embelin

The analysis of FTIR spectrum of embelin confirmed the presence of alcohols, amines and amides at the peak of 3347.31cm^{-1} . The peak at 2973.34 , 2925.99 and 1645.52cm^{-1} confirms the presence of alkanes and amino acids as a functional group, respectively. The presence of aliphatic amines,

alcohols and carboxylic acids are confirmed at the peak 1043.63cm^{-1} . The peak at 640.12cm^{-1} gives information about the presence of alkyl halide. Halogen and alkyl halide is confirmed at the peak 559.26cm^{-1} .

3.4 Water Absorption Test

When placed in liquid media, chitosan membranes can swell and retain a given water volume absorbed from the medium in their three dimensional network. In order to be used for biomedical purposes, they should absorb fluid from the body for cell transference, plus they should allow for an adequate distribution of nutrients, metabolites, and growth factors, through extracellular media. The swelling characteristics of Chitosan and Chitosan-Embelin films were evaluated by weighing the films before and after immersing in distilled water.

Table1.Absorption percentage of materials

Samples	Time period (hrs)	Initial weight (gm)	Final weight (gm)	Absorption (%)
Chitosan	0	0.033	0.033	0
	1	0.033	0.41	19
	2	0.035	0.271	87
	4	0.038	0.513	92
	24	0.045	0.993	95
Chitosan-embelin	0	0.035	0.035	0
	1	0.035	0.236	85
	2	0.027	0.436	93
	4	0.029	0.661	95
	24	0.025	0.946	97

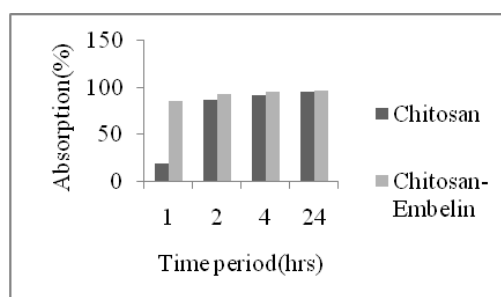


Fig 9: Graphical Representation of absorption

3.5 MTT Assay

The MTT assay is a colorimetric assay for assessing cell metabolic activity. MTT, a yellow tetrazole, is reduced to purple formazan in living cells. Tetrazolium dye assays are used to measure cyto toxicity (loss of viable cells) or cytostatic activity (shift from proliferation to quiescence) of potential medicinal agents and toxic materials. MTT assays are usually done in the dark since the MTT reagent is sensitive to light.



Fig 10: Control

3.5.1 MTT Assay of Chitosan

Table 2. Optical Density of Chitosan

Tested concentration($\mu\text{g/ml}$)	OD at 570nm (triplicate values)		
25	0.3	0.298	0.297
50	0.312	0.315	0.308

100	0.318	0.317	0.321
250	0.328	0.333	0.331
500	0.346	0.34	0.335
Control	0.312	0.300	0.306

Table3. Cell viability in Chitosan

Tested concentration (µg/ml)	% of cell viability (triplicate values)		
25	98.04	97.39	97.06
50	101.96	102.94	100.65
100	103.92	103.59	104.90
250	107.19	108.82	108.17
500	113.07	111.11	109.48
Control	100	100	100

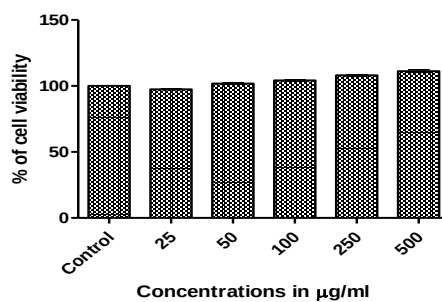
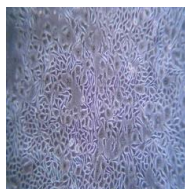
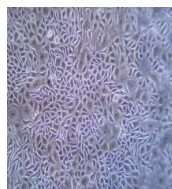


Fig 11:Cell viability in Chitosan



25µg/ml



50µg/ml

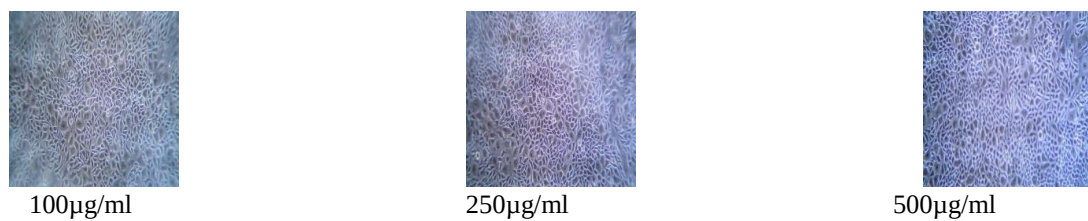


Fig 12:Cell proliferation in Chitosan material

3.5.2MTT Assay of Chitosan-Embelin

Table 4.Optical Density of Chitosan-Embelin

Tested concentration (µg/ml)	OD at 570nm (triplicate values)		
25	0.3	0.3	0.301
50	0.298	0.295	0.296
100	0.288	0.286	0.281
250	0.276	0.28	0.278
500	0.273	0.27	0.269
Control	0.306	0.312	0.300

Table5.Cell viability in Chitosan-Embelin

Tested concentration (µg/ml)	% of cell viability (triplicate values)		
25	98.04	98.04	98.37
50	97.39	96.41	96.73
100	94.12	93.46	91.83
250	90.20	91.50	90.85
500	89.22	88.24	87.91
Control	100	100	100

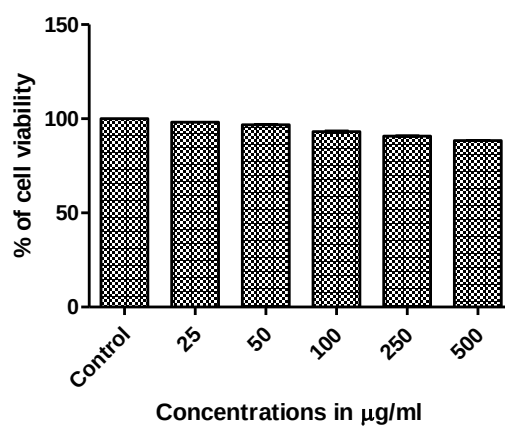
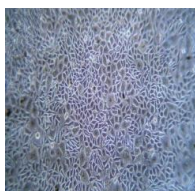


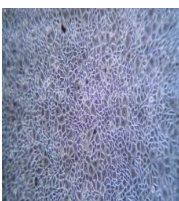
Fig 13. Cell viability in Chitosan-Embeline



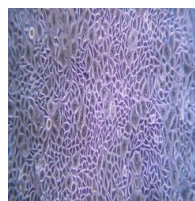
25 $\mu\text{g/ml}$



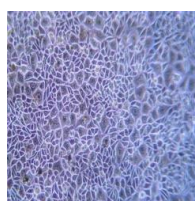
50 $\mu\text{g/ml}$



100 $\mu\text{g/ml}$



250 $\mu\text{g/ml}$



500 µg/ml

Fig 13. Cell proliferation in Chitosan-Embelin material

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