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RESEARCH ARTICLE

Wound dressing made out of Poly Vinyl Alcohol/Chitosan nanomembranes

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Abstract

Special wound care products were developed using Poly vinyl alcohol (PVA) blended with Chitosan. Electrospinning technique was adopted to produce PVA/Chitosan nanomembranes, which were used as wound dressings. PVA/Chitosan wound dressings (PCNWD) have high moisture vapour transmission property and good antimicrobial activity. PCNWD substrates do not have any cytotoxicity effects and have excellent odour absorbing capability. PVA/Chitosan wound dressing do not cause skin irritation even after 72 h of contact with the wound and the time taken for wound healing while using PCNWD is just 50% of that in the case of an open wound.

Keywords: Wound care products, poly vinyl alcohol, chitosan, cytotoxicity, skin irritation, open wound.

Introduction

Electrospinning is a technique used to process polymer fiber diameter in the range of micrometers to nanometers. Electrospinning process has increased in recent years, and this technology has been exploited for a wide range of applications. Among various nanomembrane processing techniques like drawing, template synthesis, phase separation and self assembly, electrospinning is the only method capable of producing continuous polymer nanofibres (Huang *et al*., 2003). Electrospun polymer nanofibers have very large surface area to volume ratio and flexibility in surface; these properties make the polymer nanofibers use in many important applications. Electrospun nanofibers achieve good result on properties required for wound dressings, because of its nano-size structure. Choice of wound dressings is dependent upon the severity and type of injury, as well as the stage of healing and the presence or absence of infection (McCord, 2009). The essential parameters require for wound dressing to heal wounds are absorptivity and oxygen permeability (Joshua *et al*., 2008). Additionally, ability to conform to the wound bed, provide appropriate adherence/non-adherence to the healing tissue (Zhong *et al*., 2010), provide a barrier to bacteria, enhanced moisture management , bioactivity, resorbability (Kokabi *et al*., 2007) and occlusivity are highly desirable and correlated with better outcomes. Poly vinyl alcohol (PVA) has excellent chemical resistance, physical properties, biodegradability and good fiber forming capability in electrospinning techniques. PVA polymer is suitable for blending various polymers and additives in electrospinning process to achieve uniform nanofibers (Ding, 2002). Chitosan is a polysaccharide material used to blending with PVA polymer because of its good biocompatibility (Rao and Sharma, 1997), biodegradability, cellular binding capability, antimicrobial activity and wound healing effect (Rinaudo, 2006).

In this study, PVA polymer is blend with Chitosan in different blend proportions and optimizing the electrospinning parameters using scanning electron microscope (SEM) analysis and antimicrobial activity (JIS L 1902: 2008). The optimized parameter is used for further trials and the produced nanomembrane was characterized by Fourier transform infrared spectroscopy (FTIR). The PCNWD is tested for wound dressing properties like Moisture vapour transmission rate (MVTR) (BS EN13726: 2002), cytotoxicity (ISO 10993-5: 2009), odour control capability (BS EN13726: 2002), Skin irritation and wound healing ability.

Materials and methods

Polymer and chemicals: Poly Vinyl Alcohol (PVA) with a molecular weight of 1, 24,000 g/mol, Chitosan with a molecular weight of 110,000 g/mol and acetic acid were used in this study.

Electrospinning: Electrospinning was used to produce the PVA/Chitosan nanomembranes. In electrospinning technique, there are 2 methods for the preparation of polymer solution.

Method I: In this method, the polymer is dissolved in the solvent to form polymer solution.

Method II: In this method, the polymer is heated up to its melting point and thereby the polymer solution is formed.

In this study, method I was adopted to prepare the polymer solution.

Solution preparation: The ‘n’ gram of polymer is weighed with the help of weighing balance and ‘m’ millilitre of solvent is measured with the help of micropipette.

The concentration of the polymer solution is determined by the formula:

$$\text{Concentration} = \frac{\text{'n' gram of polymer}}{\text{'m' millilitre of solvent}} \times 100\ (\%)$$

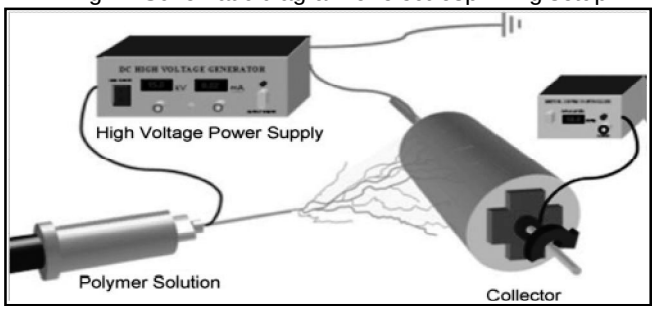
PVA solution preparation: The 'n' gram of PVA polymer salt was taken and it was dissolved in 'm' millilitre of distilled water at stirring speed of 400 rpm for 4 h at 80ºC.

Chitosan solution preparation: The 'n' gram of Chitosan was dissolved in 'n' millilitre of distilled water containing 'x%' of acetic acid to produce the Chitosan solution. The solution was stirred at 600 rpm for 3 h at room temperature.

PVA/Chitosan blend solution preparation: The PVA solution and Chitosan solution were mixed in the ratios of 80/20, 70/30, 60/40 and 50/50 at room temperature.

Electrospinning of nanomembrane: The term 'nano' represents the size of the material in 10^{-9} meter. The Polymeric nanofibers can be processed by a number of techniques like Drawing, Template Synthesis, Phase Separation, Self-Assembly and Electrospinning. Among the various processing techniques, electrospinning is the only method capable of producing continuous polymer nanofibres. Electrospinning is a unique technology that can produce non-woven fibrous articles with fiber diameters ranging from tens of nanometers to microns, a size range that is otherwise difficult to achieve by conventional techniques. A schematic representation of Electrospinning set up is given in Fig. 1. The basic setup for electrospinning consists of three major components: a high-voltage power supply, a spinneret (a metallic needle), and a collector (a grounded conductor). A high electrical supply, typically 1-30 kV, is applied to a polymer solution contained in a syringe.

Fig. 1. Schematic diagram of electrospinning setup.



The electrospinning process is governed by many parameters, classified broadly into solution parameters, process parameters, and ambient parameters. Solution parameters include viscosity, conductivity, molecular weight, and surface tension. Process parameters include applied electric field, tip to collector distance and feeding or flow rate. Ambient parameters include temperature and humidity maintained during electrospinning process. These parameters significantly affect the fiber morphology obtained. By proper manipulation of these parameters, nanofibers of desired morphology and linear density can be obtained.

Preparation and optimization of PVA/Chitosan nanomembrane: PVA/Chitosan solution was prepared by Ex-situ polymerization method. In this method, PVA solution and Chitosan solution were prepared separately and mixed in various blend proportions. Chitosan solution was prepared by dissolving chitosan powder in acetic acid. In PVA/Chitosan combination, PVA actually helps for the formation of nanofibers and Chitosan helps for quicker wound healing and new tissue generation. From initial trials, it was found that 1% chitosan solution (1% by weight of chitosan dissolved in acetic acid) is good for blending with PVA for the purpose of making nanomembrane. Hence, for bulk trials (to produce PVA/chitosan nanomembrane), 10% solution of PVA and 1% solution of chitosan were taken. The 10% PVA solution and 1% chitosan solution were blended in 4 different ratios viz. 80/20, 70/30, 60/40 and 50/50. These 4 blend solutions were electrospun to form nanomembranes. During electrospinning, basically 3 process parameters are to be optimized. They are

1. Applied voltage in kV
2. Flow rate of solution in mL/h
3. Tip to collector distance in cm.

Twenty seven optimization trials were carried out by maintaining the process parameters at different levels. The experimental design is given in Table 1.

Table 1. Experimental design for optimization of the process parameters in electrospinning.

Process parameters	Values
Applied voltage in kV	13, 15 and 18 (3 Levels)
Flow rate of solution in mL/h	0.2, 0.5 and 1.0 (3 Levels)
Tip to collector distance in cm	8, 10 and 13 (3 Levels)

Total number of experiments = 27

Table 2. Optimum process variables in electrospinning to spin PVA/Chitosan nanomembrane.

Process parameters \ Blend proportions	80% PVA / 20% Chitosan	70% PVA / 30% Chitosan	60% PVA / 40% Chitosan	50% PVA / 50% Chitosan
Applied voltage (kV)	15	13	13	18
Flow rate (mL/h)	0.5	0.5	1.0	1.0
Tip to collector distance (cm)	10	13	10	10

Optimum process parameter in electrospinning is one where uniform fibres without any bead could be produced.

Fig. 2. Electrospun PVA/chitosan nanomembrane after 4 h of processing.

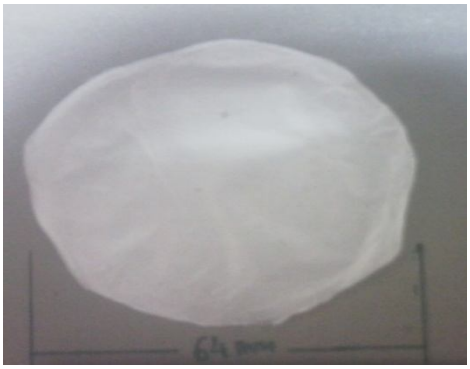


Table 3. Diameter ranges of nanomembranes produced using different blend proportions.

PVA/Chitosan blend proportions	Diameter ranges of nanomembranes
80%/20%	138 ± 48
70%/30%	176 ± 33
60%/40%	113 ± 29
50%/50%	89 ± 30

Based on the results obtained in 27 experiments, as shown in Table 1, the optimum process parameters to be maintained during electrospinning were finalized and they are given in Table 2. Fig. 2 shows the electrospun PVA/Chitosan nanomembrane with diameter of 6.4 cm obtained after 4 h of processing. Of the 4 blend proportions (PVA/Chitosan) covered in these investigations, 50/50 combination was found to give better results in terms of lower diameter of the nanomembrane (Table 3).

Antimicrobial activity of PVA/chitosan nanomembranes: The antimicrobial activity of the nanomembrane is very important for wound dressing applications. Antimicrobial test was conducted based on quantitative analysis (JIS L 1902 method using *staphylococcus aureus* (ATCC 6538) was used as the bacterial test strain. Antimicrobial activity of the nanomembranes developed in various combinations of PVA/chitosan viz 80:20, 70:30, 60:40 and 50:50 was tested as per the standard JIS L 1902. Briefly, 0.2 mL of the *S. aureus* has been inoculated on 0.5 g of each sterile test material and incubated for 18 h at 37°C. Following incubation, ratio of microbiostasis was calculated using the formula $F = M_b - M_a$ and the result was expressed in log reduction. Here F is growth value, M_b is average value of common logarithm of number of living bacteria after 18 h and M_a is average of value of common logarithm of number of living bacteria at 0^{th} h. Antimicrobial activity of the PVA/chitosan nanomembranes and 100% PVA nanomembranes are given in Table 4. The results confirm that among various blend proportions of PVA/chitosan, the nanomembranes developed using the combination of 50%/50%, resisted the bacterial growth at 56 % when compared to control (0%). Hence this combination has been chosen for further studies.

Table 4. Antimicrobial activity of PVA/chitosan nanomembrane.

PVA/chitosan nanomembrane blend proportions	% Bacteria reduction after 18 h of treatment		Remarks
	Control	Treated	
80%/20%	0	0	No activity
70%/30%	0	6	Poor activity
60%/40%	0	30	Moderate activity
50%/50%	0	56	Good activity

Control refers to nanomembranes made out of 100% PVA.

Results and discussion

Moisture vapour transmission Rate of PVA/chitosan nanomembrane: Moisture vapour transmission Rate (MVTR) is an important criterion for a wound dressing material. The liquid formed inside the wound layer first changes to vapour state and then transported to atmosphere. This moisture vapour transmission helps to heal the wound; otherwise will be wound infection. The PVA/Chitosan nanomembranes were tested for MVTR (BS EN 13726-2: 2002). A test sample of 40 mm diameter is taken and fixed over a container of 35.7 mm inner diameter, containing 20 mL of distilled water. The test sample container is weighed (W_1) before the start of the test. Then the container is kept inside an incubator for 24 h (conditions maintained inside the incubator; i) Temperature: 37 ± 1ºC and ii) RH 20%). After 24 h the container is taken out and again weighed (W_2). MVTR is calculated based on the formula:

$$X = (W_1 - W_2) \times 1000 \times 24/T$$

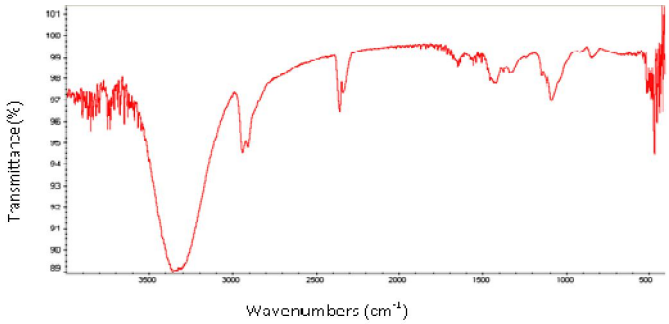
Where, X is MVTR ($g/m^2/24$ h); W_1 is the mass of the container, sample and liquid in grams; W_2 is the mass of the container, sample and liquid in grams after the test duration and T is the test period in h. MVTR values of PVA/chitosan nanomembrane samples provided in this study are given in Table 5. With increase in the proportion of chitosan in the blend, MVTR values tend to increase. This attributes to the reduction in the diameter of the corresponding nanomembrane substrates. For an infected skin, MVTR value of 2000 to 2500 is good (Lou *et al*., 2008). Hence, all the PVA/chitosan nanomembrane substrates made in this study can be considered as suitable for infected skins.

Table 5. MVTR values of PVA/chitosan nanomembranes.

PVA/chitosan blend proportions	MVTR ($g/m^2/24$ h)
80%/20%	2430
70%/30%	2560
60%/40%	2640
50%/50%	2750

Fourier-transform Infrared Spectroscopy (FT-IR) for PVA/chitosan nanomembrane: Functional groups present in PVA/Chitosan electrospun nanomembranes were studied using FTIR spectroscopy. The FTIR spectra of electrospun PVA/Chitosan nanomembrane (Blend proportion of 50%/50%) are shown in Fig. 4.

Fig. 4. FT-IR spectra of PVA/chitosan nanomembrane.



In FTIR spectra, X-axis represents wave numbers and Y-axis, the transmittance. It is discernable from the FTIR spectra, that these are peaks at i) Wave numbers in the range of 3340 to 3356 cm^{-1} (peak 1) and ii) wave number of 1733 cm^{-1} (peak 2). Peak 1 indicates the presence of –OH and –NH groups and peak 2, the presence of C=O-NH groups (Fig. 4). This in turn confirms the presence of PVA polymer and Chitosan materials in the substrates produced.

Cytotoxicity studies on PVA/chitosan nanomembrane: An *in vitro* cytotoxicity test was conducted for the PVA/Chitosan substrates using direct contact method was performed using test samples PVA/Chitosan nanomembrane as per (ISO 10993-5). In this method, a monolayer culture of L-929 is used as the medium. Test samples, negative and positive controls were placed on the medium. After incubation at 37 ± 1ºC for 24 h, the monolayer medium is examined microscopically for the response around the test specimen. The reactivity levels observed are graded as 0, 1, 2, 3 and 4 based on zone of lysis, vacuolization, detachment and membrane disintegration as explained in Table 6.

Table 6. Grading of cytotoxicity.

Reactivity	Description of reactivity zone	Grade
None	No detectable zone around or under sample	0
Slightly	Some malformed or degenerated cells under specimen	1
Mild	Zone limited to area under specimen	2
Moderate	Zone extending specimen size up to 0.33 cm	3
Severe	Zone extended farther than 0.33 cm beyond specimen	4

As per ISO 10993-5, reactivity grade higher than 2 is considered as cytotoxicity. The PVA/chitosan nanomembrane substrates produced in this study (50%/50%) were found to have “None” reactivity and hence graded as “0” as far as cytotoxicity is concerned.

Fig. 5a. Diethylamine Vs time in control specimen.

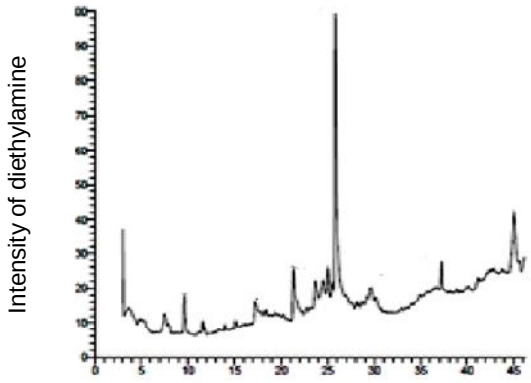
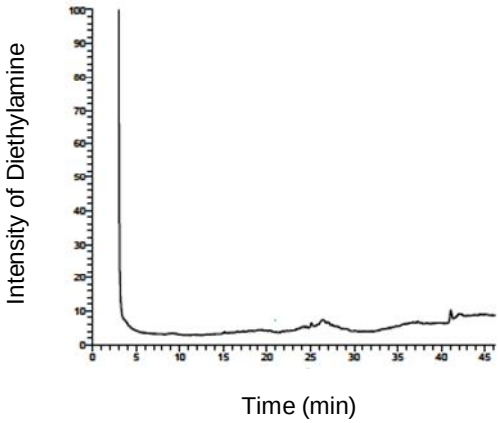


Fig. 5b. Diethylamine Vs time in PVA/chitosan nanomembrane.



Odour control ability of PVA/chitosan nanomembrane: The objective of the test is to assess the resistance of wound dressing to penetration by odour. The sample is tested as per BS EN 13726-6: 2003 method. A stainless steel container of 35.7 mm inner diameter and 40 mm height is used for carrying out the test. The test consists of 2 parts.

Part I: Here, the container is heated for 1 h at 105ºC. Then, nitrogen gas is purged inside the container. After this 0.5 µL of pure diethylamine is also passed inside the container and the container is kept inside an incubator at 37ºC for 20 min. After 20 min, 250 µL sample of nitrogen gas is removed from the container and injected into GC (Gas Chomatography) instrument. The GC instrument estimates the intensity of diethylamine present in nitrogen gas and provides the information in the form of a graph (Fig. 5a). In the graph, the intensity of diethylamine present in Nitrogen gas is plotted against time.

Part II: The container is heated for 1 h at 105ºC. After this, a 1.3% w/v solution of diethylamine (0.26 g of diethylamine dissolved in 20 mL of distilled water) is added into the container.

Table 7. Wound healing in open wound and PVA/chitosan nanomembrane treated wound.

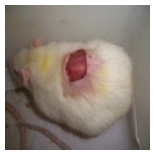
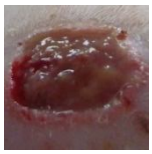
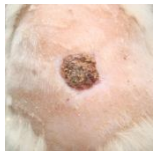
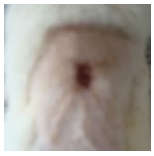
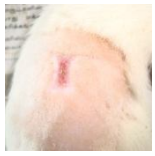
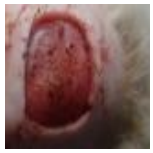
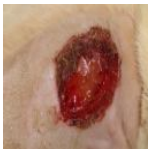
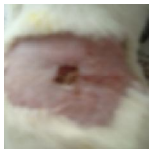
Type of wounds	Extent of Wound healing					
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
Open wound						
PVA/chitosan nanomembrane						

Table 8. Weight of the traced portions of wounds on different days.

Type of wound	Weight of traced portions of the wound (g)					
	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
Open wound	100	154.89	110.78	82.44	40.72	11.23
PVA/chitosan nanomembrane treated wound	100	56.99	19.6	0	-	-

Then, the test specimen is kept inside the container, nitrogen gas is purged inside the container and the container is kept inside an incubator at 37ºC for 30 min. After 30 min, 250 µL sample of nitrogen gas is removed from the container and injected into GC (Gas chomatography) instrument for the analysis of intensity of diethylamine in nitrogen gas. The information is given in Fig. 5b. In the graph, the peak shown at 25-27 min represents diethylamine. The diethylamine present in PVA/chitosan nanomembrane is just 10% of that exhibited by control specimen. Hence, PVA/chitosan nanomembrane has excellent odour absorbing capability.

Skin irritation potential of PVA/Chitosan nanomembrane: PVA/chitosan nanomembrane samples were evaluated for potential skin irritation, when they are used for covering the wound. The evaluation was as per ASTM F 719-81 standards. The test method is explained in Appendix I. This study was carried out after approval from Kovai Medical Centre Ethical Committee on the use and care of experimental animals (Kovai Medical Centre Ethical Test/ Ministry of Textiles Research Studies/ Ethical test no: KMCRET/ MOTRS/01/2012-13). Six healthy rabbits were selected for the study and PVA/Chitosan wound dressings were applied on the 6 rabbits. The study has shown that PVA/chitosan wound dressing do not causes any skin irritation even after 72 h of contact with the wound.

Wound healing ability of PVA/chitosan nanomembrane: The extent of wound healing provided by a given wound dressing was evaluated using the method proposed by Morton and Malone (1972).

As per this method, 12 healthy rats were employed for the experimentation and they were separated into 2 groups (Group I and Group II) each with 6 rats. Excision of wounds was made on the rats as per the method suggested by Morton and Malone. The rats were anaesthetized with anaesthetic ether and placed in operation table in their natural position. A square wound of about 1.5 cm (width) x 0.2 cm (depth) was made on depilated ethanol-sterilized dorsal thoracic region of rats. Infection was made on wounds by *staphylococci aureus*.

1. Group I rats were left as they were without application of any wound dressing (open wound).
2. Group II rats were treated using PVA/Chitosan nanomembrane wound dressing.

The dressings were applied on the wounds of the rats every day till the epithelialisation was complete. The extent of wound contraction was studied by tracing the raw wound area in a tracing paper on 0^{th} d, 7^{th} d, 14^{th} d, 21^{th} d, 28^{th} d and 35^{th} d. The weights of the traced portions of the wounded area of rats subjected to different treatments were measured using electronic balance. Based on the difference in weight, the superiority or otherwise of a particular wound dressing is determined. Table 7 shows, the extent of wound healing noticed while using different wound dressings on different days. The weights of the traced portions of open wound and PVA/chitosan nanomembrane treated wound on different days are shown in Table 8. It is clear from Table 8, that there is a decrease in wound area with the application of PVA/chitosan nanomembrane wound dressings. From this study, the nanomembrane was helps to heal the wounds very quickly because of its high surface area to volume ratio.

Conclusion

SITRA has developed special type of wound dressing viz.) PVA/chitosan nanomembrane wound dressing (PCNWD). PCNWD is found to be ideally suited for infected skins in view of their high moisture vapour transmission property (MVTR) and PCNWD Substrates have good antimicrobial activity. PCNWD do not have any cytotoxicity effects and PCNWD samples exhibited excellent odour absorbing capability. PVA/chitosan substrates do not cause any skin irritation even after 72 h of contact with the wound. The time taken for wound healing while using PCNWD is around 50% lower as compared to that in the case of an open wound.

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Appendix I

Standard test method for assessing skin irritation (ASTM F 719-81 standard)

Principle of measurement: Exposure of skin to the test material is accomplished by means of a patch test technique employing 2 intact sites on the back of each of 6 albino rabbits. The skin is clipped free of hair 1 d prior to testing. The test substance is applied using 0.5 mL for liquids, 0.5 g for solids or semisolids and a 2.5 by 2.5 cm square patch for films. After application, each test site is covered with 2.5 by 2.5 cm gauze flat and entire trunk is occluded with a polyethylene sleeve. After 24 h the sleeve, flat and test material are removed and test sites are evaluated for erytheme and edema.

Scoring method: Using the criteria given in Table 9, the test sites are scored for Erythema (ER) and Edema (ED). Test sites can also be scored for erythema and edema at 48 h as well as 72 h after removal (as per the usage requirement) using the criteria given in Table 9.

Table 9. Skin reactions score for Erythema and Edema formations.

Reaction	Description	Score
Erythema (ER)	Erythema and Eschar	
	No erythema	0
	Very slight erythema (barely perceptible)	1
	Well-defined erythema (pale red in colour)	2
	Moderate to severe erythema (red and area well defined)	3
	Severe erythema (beet redness to slight eschar formation)	4
Edema (ED)	Edema formation	
	No edema	0
	Very slight edema (barely perceptible)	1
	Slight edema (edges of area well defined by definite raising)	2
	Moderate edema (edges raising approximately 1 mm)	3
	Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4