Available online at www.ijournalse.org

Emerging Science Journal

(ISSN: 2610-9182)

Vol. 7, No. 3, June, 2023

Characteristics and Antibacterial Properties of Film Membrane of Chitosan-Resveratrol for Wound Dressing

Basri A. Gani [1*], Nur Asmah [2], Cut Soraya [3], Dharli Syafriza [4], Sri Rezeki [5], Muhammad Nazar [6], Subhaini Jakfar [7], Nurtami Soedarsono [8]

[1] *Department of Oral Biology, Dentistry Faculty, Universitas Syiah Kuala, Darussalam, Banda Aceh, Aceh, Indonesia.*

[2] *Department of Conservative Dentistry, Dentistry Faculty, Universitas Muslim Indonesia, Makasar, Sulawesi Selatan, Indonesia.*

[3] *Department of Conservative Dentistry, Dentistry Faculty, Universitas Syiah Kuala, Darussalam, Banda Aceh, Aceh, Indonesia.*

[4] *Department of Pediatric Dentistry, Dentistry Faculty, Universitas Syiah Kuala, Darussalam, Banda Aceh, Aceh, Indonesia.*

[5] *Department of Oral Medicine, Faculty of Dentistry, Universitas Syiah Kuala, Darussalam, Banda Aceh, Aceh, Indonesia.*

[6] *Department of Chemistry, Education Faculty, Universitas Syiah Kuala, Darussalam, Banda Aceh, Aceh, Indonesia.*

[7] *Dental Material Department, Dentistry Faculty, Universitas Syiah Kuala, Darussalam, Banda Aceh, Aceh, Indonesia.*

[8] *Department of Oral Biology, Dentistry Faculty, Universitas Indonesia, Salemba, Jakarta, Indonesia.*

Abstract

The research aimed to evaluate the film membrane of Nano Chitosan Resveratrol (NCHR) for biological, physicochemical, and antibacterial properties. Psychochemically, the functional groups of chitosan compounds were examined by FTIR, chemical compounds by GCMS, and the morphology of chitosan and chemical elements by SEM-EDS. Biologically, the characteristics of NCHR were examined by solubility, swelling, permeability, and biodegradation tests. Meanwhile, the antibacterial properties were examined for inhibition of *Porphyromonas gingivalis* (*P. gingivalis*) ATCC 33277 by Minimal inhibition concentration (MIC) and growth assessment by spectrophotometry. Nano Chitosan (NCH) has appeared at 1033.85 cm^{-1} as a sharp peak indicating the P=O group and contains anti-toxicity compounds (Ethane, 1,1-diethoxy- (CAS) 1,1-Diethoxye) is 81.06% and antioxidant compounds Limonene is (1.28%). In addition, NCH has chemical elements, Oxygen Weight (69.4%), calcium (19.7%), magnesium (6.6%), and phosphorus (4.3%). NaCl 0.9%, PBS, and Aquades. In addition, it has an excellent index of water vapor transmission rate (WVTR) in all solvents ($R^2 \geq 0.95$). The NCHR membrane film is bacteriostatic (≤ 300 CFU/mL) with each value of Minimal Inhibition Concentration (MIC) >15 mm. The Nano chitosan contains antitoxic, antioxidant, and antibacterial compounds with high oxygen elements. The film membrane of nano chitosan resveratrol can maintain the stability of changes in pH with a very high solubility index, swelling index, and WVTR index, as well as good biodegradation and antibacterial properties.

Keywords:
Antibacterial;
Biodegradation;
Film-membrane;
Nano chitosan;
Resveratrol;
Wound Dressing.

Article History:

Received:	23	November	2022
Revised:	10	February	2023
Accepted:	19	March	2023
Available online:	10	May	2023

1- Introduction

Health technology advances in tandem with the evolution and modification of disease patterns. So that it draws the attention of the world's researchers to act and find solutions to these health issues [1]. Several health research fields currently synergize with other areas, especially those relevant to medical engineering or pharmacy. Now, medical engineering and pharmacy research mainly focus on the product of tools or materials that help treat disease prevention

* **CONTACT**: basriunoe@unsyiah.ac.id

DOI: http://dx.doi.org/10.28991/ESJ-2023-07-03-012

and treatment [2]. Sensor-based research, or the breeding of natural materials, is highly encouraged by researchers across disciplines. In addition to abundant raw materials, it is also cheap and easy to obtain [3]. One of them is a crab shell (*Scylla paramamosain*) as a raw material for synthesizing chitosan for health purposes in various application dosage forms.

Chitosan is one of the natural polymers obtained from the deacetylation of chitin. It has several advantages, such as biocompatibility, biodegradability, non-toxicity, and a chelating agent, bacteriostatic and fungistatic properties [4]. The use of chitosan as a source of medicinal raw materials has been widely reported. In general, chitosan is used as a drug delivery agent in capsules and films [5]. Various strategies have been made to increase the oxidation power and penetration of the chitosan film's pharmacodynamic and pharmacokinetic properties, including making it a nano-sized joint to improve the absorption properties at the application site [6]. Among them are adding other active ingredients (resveratrol) such as Moringa oleifera plants to increase antioxidant, antibacterial, anti-inflammatory, and anti-thrombotic. Arévalo-Híjar (2018) reported that *M. oleifera* has very high antioxidant and antibacterial properties [7].

The addition of resveratrol (*M. oleifera*) is expected to increase the response of the chitosan film to the environment and increase its workability during the reaction to postoperative wound healing or capsule coating preparations for drug delivery. Adding other medicinal ingredients to the chitosan film with non-synthetic (natural) sources, physicochemically and biologically, provides strength, biocompatibility, and good biodegradation [8]. Using base films such as Carboxy ethyl Cellulose (CMC) can also increase the solubility and permeability of the membrane to water sorption. In addition, it can increase the tensile strength, reduce structural changes in chitosan, and decrease the solubility of the film in the air [9].

Changes in wound pH and temperature cause an increase in pathogens, thereby retarding wound healing and causing a secondary infection. Antibiotics are said to prevent the development of pathogens, but they do not hasten wound healing and tend to promote pathogen resistance. Applying a film membrane composed of natural materials like chitosan to the surface of the surgical wound before suturing is anticipated to offer protection against oxidative stress on cells and tissues during the healing phase, prevent the growth of bacteria in the wound area, and enhance cell and tissue proliferation [10]. It has been reported that chitosan films containing antioxidants promote wound healing, prevent secondary infection, and increase the supply of nutrients to tissues and cells undergoing repair during the healing phase [11].

The biological and physicochemical properties of film products containing natural ingredients can determine the pharmacokinetic and pharmacodynamic properties of drugs in films during adaptation to the intracellular and extracellular fluid environments in the wound healing process [12]. One of the functions of chitosan films containing antioxidants is to inhibit the growth of infectious agents (bacteria and fungi), thereby promoting wound healing [13]. In addition to serving as a chelating agent, chitosan's biocompatibility permits the delivery of active medicinal compounds, such as antioxidants for health purposes, in capsule form as a drug delivery or wound dressing film [14].

The resveratrol (*M. oleifera*) to the chitosan base is expected to reduce the development of pathogens in the postoperative wound area, increase cell proliferation, and repair surrounding tissues. The antioxidant properties of *M. oleifera* can maintain the balance of changes in the chitosan film from being affected by changes in pH, solubility, and temperature. So, it is hoped that the chitosan film added with *M. oleifera* extract can work based on the wound healing phase and postoperative bone repair [15]. Ervolino et al. (2019) reported that an increase in cell proliferation and a decrease in inflammatory cells, as well as a reduction of osteonecrosis in bone, are a series of phases of healing [16]. The use of chitosan membrane films is expected to be involved in the framework of wound healing. In vitro tests such as physicochemical, biological, and antibacterial properties are a long series of tests for the possible use of chitosan membrane films for health purposes.

This study created membrane films for medical applications from nano crab shell chitosan containing resveratrol. It is used to promote postoperative wound healing and prevent secondary infections. This study aims to evaluate the film membrane of nano chitosan-resveratrol in terms of its physicochemical, biological, and antibacterial properties. As a result, it is anticipated that continuous information will be available to support future tests on the film membrane of nano chitosan-resveratrol that will be applied to medical applications.

2 - Material and Methods

2-1- Material

This study used chitosan material from crab shells (*Scylla paramamosain*), which were obtained at point coordinates (5.605783038489495, 95.34665240501441), while *Moringa oleifera* was obtained at coordinates (5.585492189676038, 95.35057255285955). Both of these materials are stored in the Oral Biology Laboratory, Faculty of Dentistry, Syiah Kuala University, Darussalam, Banda Aceh, Indonesia.

2-2- Research Procedure

This study evaluates the biological and chemical properties of chitosan nano-membranes derived from crab shells and mixed with natural resveratrol (*M. oleifera*) for health purposes (wound healing). The treatment begins with the production of chitosan nano-powder and the extraction of Moringa leaf [17]. Based on nano chitosan's morphology and chemical elements, nano chitosan was analyzed for chitin and chitosan groups physicochemically, and chemical compounds were also investigated [18]. In addition, chitosan nano-membranes were manufactured, characterization tests, including solubility, swelling, WVTR, and pH measurements, were conducted, and biodegradation and antibacterial tests were required. The flowchart for the research is shown in Figure 1.

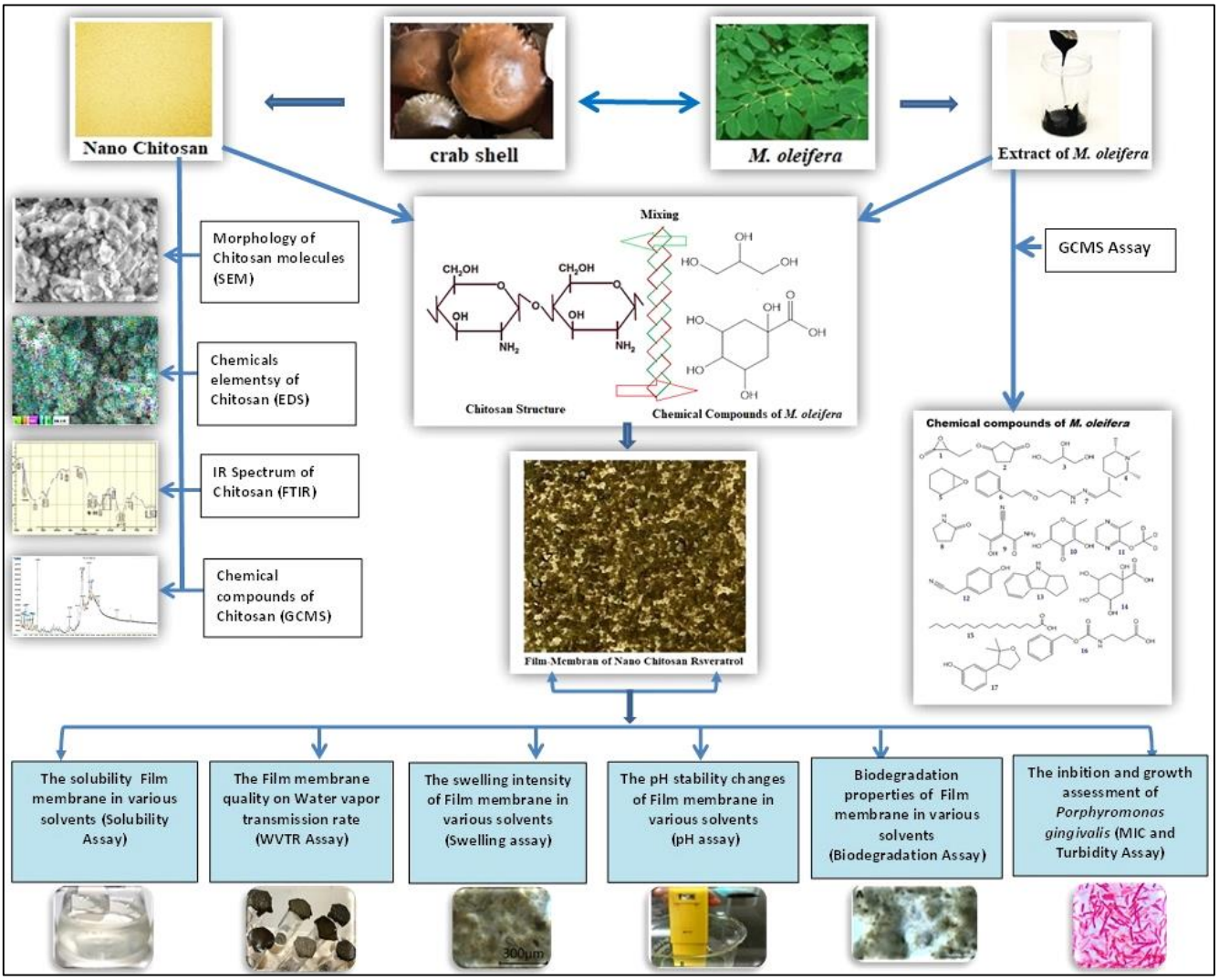


Figure 1. The flowchart of the research methods

2-3- Production and Isolation of Chitosan

A total of 7 kg of crab shells were separated from the meat, washed, dried in the sun for five days, and pounded until smooth. The crude material is then blended and crewed (100 mesh). The results passed through this sieve are used to obtain chitin. Isolation of Chitosan begins with demineralization, deproteination, and deacetylation (the transformation of Chitin into Chitosan) [19]. In the demineralization stage, the preparation of crab shell flour weighed as much as 100 grams, and then 1 N HCl was added (ratio 1:12 w/v). Then stirred at 75 °C for one hour, the residue was filtered and washed with distilled water until neutral, and then dried at 75 °C for 24 hours in the oven. In the deproteination stage, the demineralized solid was added to 2 N NaOH (ratio 1:6 w/v), stirred, and then heated at 80 °C for one hour. The filtrate is then removed by filtering. The residue (Chitin) was washed with distilled water until neutral and dried at 75 °C for 24 hours in an oven. In the deacetylation stage (transformation of chitin into chitosan), chitin was boiled in 50% NaOH solution (1:5 w/v) at 90 °C for 120 min (stirring). The solid is separated from the liquid by filtering. The residue (Chitosan) was washed with water until neutral and dried at 80 °C for 24 hours in the oven. The resulting chitosan was then weighed, obtaining as much as 12,097 grams.

2-4- Extraction of Plant Material

1 kg of *Moringa oleifera* leaves are harvested and washed. Two days of drying until wilted, then 48 hours in a 50 °C oven. Moringa leaves are blended into a powder. Moringa leaf powder is stored airtight. The powder is soaked in 100 mL of 96% ethanol in a clean, flat-bottomed glass container. Changing the solvent for three days remade the residue and filtrate. The filtrate is concentrated with a rotary vacuum evaporator at 50 °C and 75 mmHg to obtain the extract [20]. Shimadzu Japan gas chromatography QP2010PLUS with a fused GC column (2010) covered with polymethyl silicon was used for GC-MS analysis of *M. oleifera* ethanol extract and chitosan. Conditions: 80–200 °C, 5 °C/min, 200 °C for 20 min. The FID temperature was 300 °C, the injection temperature was 220 °C, and the nitrogen carrier gas flow was 1 mL/min, a 1:75 split. 116.9 kPa. 30 m column, 0.25 mm diameter, 50 mL/min flow rate [21].

2-5- Preparation of Nano Chitosan

Manufacture nano chitosan using a ball milling tool (Fritsch Pulverisette Classic Line Planetary Ball Mill, Germany). In the first stage, a stainless ball is placed at the bottom of the vial, after which chitosan powder is poured and milled into a nano (50-gram sample). Next, the seal ring is installed in the correct position, and then the vial is closed and placed on the planetary ball mill. Next, the lock is placed on the vial cap, tightened to the maximum, the lock lever is closed, and the vial is centrifuged at 450 xg for 40 hours. Then the ball mill is rotated every hour with a 15 min delay. Then press "start" and let planetary work according to the settings made until finished.

2-6- Observation of the Morphology of Chitosan and Chemical Elements

Scanning Electron Microscopy-Energy Dispersive X-Ray Spectroscopy (ThermoFisher, Netherlands) was used to examine nano chitosan's morphology and chemical properties. The sample is placed in a vacuum chamber, and the sample height must follow the calibration standard. Then the appliance is turned on with a power of 20 kV. The sample is shifted slowly to get the area to be photographed on the SEM screen. Brightness, contrast, and focus are adjusted until a good picture is obtained. Photographs are taken at 500x, 1000x, 1500x, and 2000k magnifications. The procedure for testing samples with EDS is as follows: The area to be analyzed has been determined. Data retrieval is carried out by scanning a scanner on the EDS device, and data will be obtained within 1 second. The results obtained can be viewed on the EDS screen. The type and number of elements contained in the scan area will be confirmed using the EDS database software [22].

2-7- Analysis of FTIR Spectrum of Nano Chitosan

The chemical functional groups of nano chitosan were assessed using Fourier Transform Infrared (FTIR) with a wave number of 4000 cm^{-1} - 400 cm^{-1} and transmittance spectra. In the first stage, the sample is placed on the surface of a transparent infrared prism with a refractive index that is always higher than the sample (the refractive index of bacterial cells is estimated at 1.39). The radiation beam is directed at one of the prism walls for the prism-sample interface at an angle higher than the barrier. Under these conditions, complete reflection occurs on the side of the internal prism. The reflected light exits through the walls of the second prism, where the light intensity and absorption spectrum are recorded [23].

2-8- Production Film-membrane of Nano Chitosan Resveratrol

Based on Hasan et al. (2020) research, membrane films are manufactured using Carboxymethyl cellulose (CMC) as the basis for film adhesives) [18]. The CMC and Chitosan were both weighed at 4.8 grams and weighed and dissolved in 400 mL of distilled water until a gel formed. Chitosan was dissolved in 50 mL of 1.5% CH3COOH until a gel formed. The CMC solution and Chitosan solution were mixed and stirred until homogenous for 15 min, then 4.8 grams of glycerol was added and stirred until homogeneous for 30 minutes. Then resveratrol Moringa oleifera was added with variations of 10%, 20%, and 30% (1.6 grams, 3.2 grams, and 4.8 grams). Then the solution was stirred for 30 min until homogeneous. The film solution was poured over the mold and left at room temperature for three days (until dry). The film is removed from the mold, and a characterization test is carried out.

2-9- Characteristic Assay of Film-membrane of Nano Chitosan Resveratrol

Test the characteristics of the NCHR membrane film using the working principle of Hasan et al. (2020) [18]. Solubility Test. The film was cut to 1 × 1 cm and weighed for initial data. Then, the film was inserted into an Erlenmeyer containing 20 mL of solvent (DMSO: Solarbio Science and Technology, Beijing Chine), NaCl0.9% (Emjebe Pharma, Surabaya, Indonesia), PBS (Thermo Fisher, Swedia), and aquadest (Purification in our laboratory). Then the film was stirred for 15 minutes, allowed to stand for 24 hours, and dried at 105 °C for 8 h. Then the film is weighed to obtain final data. Solubility formula (%) = $\frac{W_i - W_f}{W_i} \times 100\%$ (W_i = initial weight W_f = final weight.

Swelling Assay. The film was cut to 1 x 1 cm and weighed dry, then put into DMSO, NaCl 0.9%. PBS and aquadest, respectively 0.15 mL. Then soaked for 2 h, the film was removed, and the surface was dehydrated. It is then weighed in a moist (wet) state. Swelling Degree Formula = $\frac{Mw-Md}{Md}$ (M_w = film weight in wet condition; M_d = dry film weight).

Water Vapor Transmission Rate (WVTR) Assay. A total of 5 mL of DMSO solvent, 0.9% NaCl. PBS and aquadest were put in different test tubes, and then the film was cut according to the size of the mouth of the test tube used (d = 1.1 cm). Then the film is glued to the mouth of the test tube. Then the test tube that has been glued to the film is weighed. Next, the test tube was put into a desiccator filled with silica gel. Then the test tubes were considered every 24 h for five days. The WVTR results have then graphed the relationship between mass and time and calculated using the formula: WVTR = $\frac{\Delta W}{A.\Delta t}$ (Δw = change in film weight after 24 hours; Δt = Times (24 h); A = film area (m^2)

pH Assay. The NCHR membrane film was cut 1x1 cm, then dissolved in 5 mL of different solvents (DMSO, 0.9% NaCl, PBS, and Aquadest) for 24 h. Then vortex three times for 5 min. Then the pH of the solution was checked with a pH meter (Mettler Toledo, Switzerland).

2-10-Biodegradation Assay

NCHR membrane films of different concentrations were cut 1×1 cm and then incubated at 37 ^oC for 3 minutes. Then the film was placed in a test bottle and tightly closed for 15 min. Furthermore, each bottle containing the film was inserted with a different solvent (DMSO, 0.9% NaCl, PBS, and aquadest) through the bottle wall, each 5 mL. The relegation assessment uses 1 min, 2 min, 4 min, 6 min, 8 min, and 15 min. Quantitative data were examined by spectrophotometry based on the solubility of the film (turbidity) OD 600 nm. Meanwhile, qualitative data were analyzed using a stereo microscope with a bar scale of 200 µm.

2-11-Antibacterial Assay

The oral bacteria used in the study included an aerobic bacterium, *Porphyromonas gingivalis* ATCC 33277. The bacterial preparation was calibrated with Mc. Farlan 0.5 ($1.5x10^8$) or OD 0.08-0.1 (≤300 CFU/mL) [24]. The antibacterial test of the film was carried out from two perspectives. The first was based on the colony's taste buds using the disc method, and the second was the quantity of bacterial growth examined by spectrophotometry.

The membrane film is made to resemble discs (6 mm). Furthermore, culture preparation of 30 µL of bacteria was carried out in Mueller Hinton Agar medium (Merck KGaA, Darmstadt, Germany) and spread (streak) throughout the medium. Subsequently, membrane film discs were placed based on the respective concentrations of resveratrol and incubated for 24 h and 48 h. The clear zone area was measured using a caliper with vertical and horizontal directions, which became the antibacterial value of the membrane film.

Bacterial growth was evaluated using a base film soaked in various solvents (DMSO, 0.9% NaCl, PBS, and Aquadest). The film was softened in a different solvent for 30 min in the first stage. Then vortexed for 5 minutes. It was repeated two times until the film was lysed and blended with the solvent. Then it was centrifuged at 3000 xg for 10 min. The supernatant was collected, then 1 mL of solvent with 100 µL of bacterial suspension was added to a 2 mL vial. It was then incubated in an aerobic atmosphere at 37 ^0C for 24 h. The film residue was separated by centrifuging 3000xg for 10 min, and the supernatant was considered living bacterial cells. Furthermore, it was measured by spectrophotometry with a wavelength of 600 nm [25].

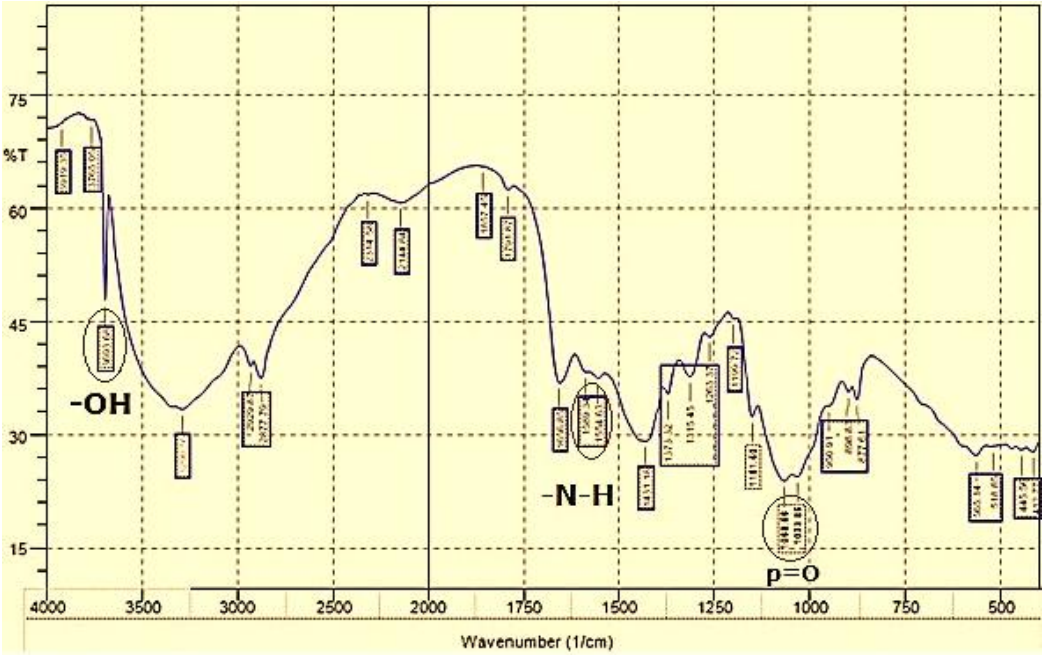
2-12-Statistical Analyses

Data on membrane film pH, biodegradation, and antibacterial properties were analyzed by One-Way ANOVA, with a significance limit of $p<0.05$.

3- Results and Discussion

3-1-FTIR Analysis of Chemical Group of Nano Chitosan

Figure 2 shows that FTIR provides information about the functional properties of nano chitosan from crab shells that link functional groups and their structures. Figure 1 shows that nano chitosan peaks at a wave number of 3290.56 cm^{-1} showing stretching vibrations of the –NH2 and –OH groups. This area's peak width between 3919.35-3290.56 cm^{-1} is associated with the hydrogen bond strain vibration (O-H) [26]. In contrast, the stretching NH overlap of the primary amine and amide-type occurs in the same region [27]. The peaks formed at 1656.85 cm^{-1} and 1554.63 cm^{-1} indicate an interaction between the NH+3 group. Silverstein reported that wave number 1650-1580 cm^{-1} showed the presence of a functional amine NH primary group with a bending vibration type [28]. The wave number of 1151.50 cm^{-1} indicates C-O-C, while the IR spectrum of chitosan nanoparticles appears at 1033.85 cm^{-1} as a sharp peak indicating the P=O group.



Peak	Intensity (%)	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Group
910.91	33.81	0.35	954.76	918.12	16.63	0.15	P=O bending
1033.85	24.40	1.51	1045.42	956.69	48.86	1.29	
1040.56	23.88	3.05	1136.07	1047.35	50.81	2.90	
1151.50	32.41	4.48	1190.08	1138.00	22.40	1.21	C-O-C stretching
1554.63	37.44	0.78	1577.77	1539.20	16.28	0.17	-N-H bending
1589.34	38.28	0.69	1616.35	1579.70	14.95	0.14	
1656.85	36.87	9.25	1776.44	1618.28	48.68	3.76	
3290.56	33.38	1.36	3332.99	2989.66	150.20	4.24	-NH2 and -OH stretching
3693.68	47.82	16.23	3753.48	3676.32	15.42	2.11	
3765.05	71.63	0.18	3784.34	3755.40	4.17	0.01	
3919.35	71.31	0.11	3923.21	3900.07	3.36	0.00	

Figure 2. FTIR spectrum of cellulose, chitin, and chitosan in the 4000-500 cm^{-1} wavenumber region (above). The transmittance (%T) value of nano chitosan of crab shell

Silverstein et al. (2005) reported that the P=O functional group with stretching vibration was found at 1040–910 cm^{-1}. The formation of the P=O active group indicates cross-linking between the phosphate group and chitosan through ionic bonds. This analysis's results suggest that a nanoparticle solution has been formed [28]. So, it is expected that adding resveratrol can improve the mechanical properties of naturally brittle chitosan and increase the formation of ionic cross-links between chitosan molecules. This method has been reported to enhance nano materials' mechanical properties, thermal properties, barrier properties, and physicochemical properties compared to microscale materials [29]. Chitosan becomes more stable and can increase the adsorption capacity of tissues and cells [30]. This property will provide advantages in applying nano chitosan as an edible coating material that can be used as drug delivery for tissue and cell repair after surgery.

3-2- Chemical Compound of Nano Chitosan

Table 1 and Figure 3 show several chemical compounds in nano chitosan of crab shell, such as Ethane, 1,1-diethoxy- (CAS) 1,1-Diethoxye (81.06%). Diethoxye is reported to have antitoxic and anti-inflammatory functions while increasing the response to infectious agents [31]. In addition, diethoxy is also reported as an antitoxin [32]. In addition, it contains several antioxidant compounds, such as dl-limonene, which functions as an antioxidant, antibacterial, and anti-inflammatory [33, 34]. Terpene d-Limonene has tissue repair properties for wound healing and angiogenesis around scars, and it promotes tissue regeneration, according to d'Alessio et al. (2014). d-Limonene has notable anti-inflammatory effects on skin inflammation and wound healing [35]. Chitosan contains active compounds that prevent tissue toxicity and increase intracellular fluid balance during wound healing [36]. In addition, it has been reported that butane,1,1-diethoxy-3-methyl compounds are involved in embryonic angiogenesis and wound healing [37]. It is believed that the active compounds in chitosan accelerate wound healing by stimulating inflammatory cells, macrophages, and fibroblasts, thereby shortening the inflammatory phase and accelerating the onset of the proliferation phase [38].

Table 1. GCMS evaluation of chemical compounds of nano chitosan from crab shell

Peak#	R.Time (Minute)	Area	Area%	Height	Height%	A/H	Compound Name
1	3.121	38013762	81.06	3234129	57.24	11.75	Ethane, 1,1-diethoxy- (CAS) 1,1-Diethoxye
2	3.619	1549648	3.3	311708	5.52	4.97	Propane, 2,2-diethoxy-
3	3.78	2621077	5.59	342545	6.06	7.65	3-Penten-2-one, 4-methyl-
4	4.279	512270	1.09	140926	2.49	3.64	2-Pentanone, 4-hydroxy-4-methyl- (CAS)
5	4.846	699781	1.49	153877	2.72	4.55	Propane, 1,1-diethoxy-2-methyl-
6	6.311	1354065	2.89	365800	6.47	3.7	Butane, 1,1-diethoxy-3-methyl-
7	7.484	602583	1.28	231786	4.1	2.6	dl-Limonene
8	13.238	108317	0.23	64703	1.15	1.67	trans-Caryophyllene
9	13.741	117591	0.25	46106	0.82	2.55	.alpha.-Patchoulene
10	14.278	135772	0.29	75691	1.34	1.79	Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-di
11	16.04	1179038	2.51	682799	12.08	1.73	1(2H)-Naphthalenone, octahydro-4a,8a-dim

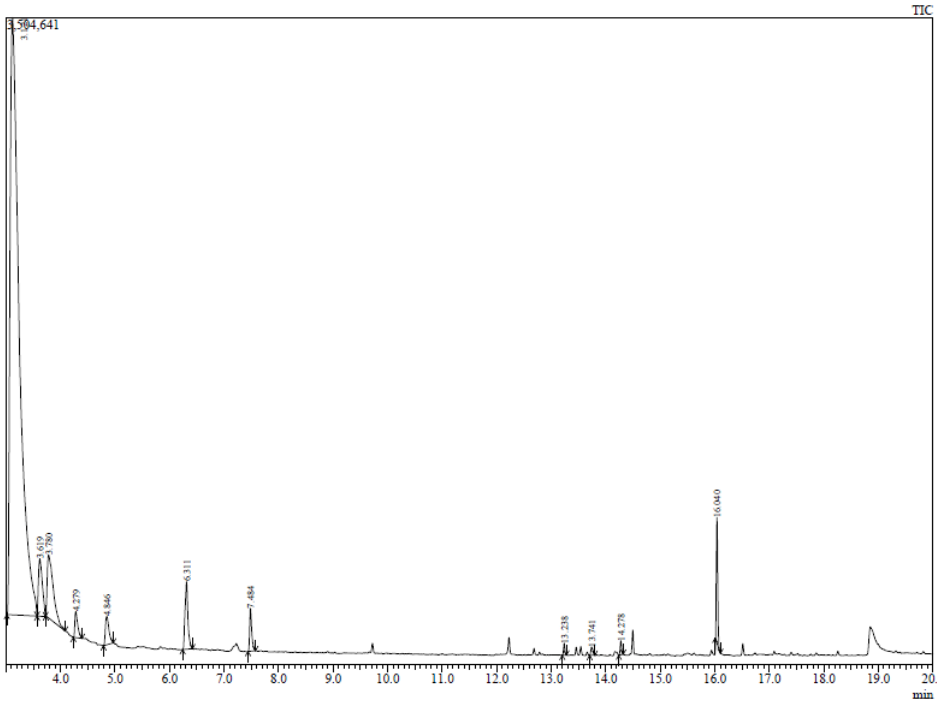


Figure 3. GC spectrum of crab shell nanochitosan. Peak starts from retention of 3.121 min to 16.04 min

Applying nano chitosan in the form of membrane films is anticipated to improve the physiological stability of cells and tissues during the wound healing phase by acting as a drug delivery agent. In addition to increasing the absorption and penetration of active ingredients in the wound area, adding polyphenols from natural resveratrol to the chitosan film helps organize the formation of collagen fibers, fibroblasts, and inflammatory cell responses to reduce cell oxidative activity [39]. Stricker-Krongad et al. (2018) reported that chitosan-based wound dressings could accelerate the clotting process. In addition, the wound-healing properties of chitosan allow for the safe and effective bandaging of excision wounds for hemostasis [40].

3-3- Morphological and Chemical Elements of Nano Chitosan

Chitosan is one of the most researched biopolymers for wound healing due to its biocompatibility, biodegradability, non-toxicity, and antimicrobial properties. In addition, chitosan and its derivatives have garnered significant interest due to their ability to expedite wound healing and be processed into various forms (gels, membranes, and films) [41]. These characteristics make chitosan-based materials highly adaptable and promising as wound dressings. In addition, labeling with secondary natural metabolites (resveratrol) can act as an antimicrobial, anti-inflammatory, and healing stimulant [42]. Based on the morphology of the chitosan-resveratrol film membrane, chitosan's function as a polyphenol delivery agent in Moringa leaves to enhance wound absorption is demonstrated. The morphology of the film membrane of nano chitosan mixed with resveratrol (*M. oleifera*) is depicted in Figure 4, indicating that the active component of resveratrol is located on the surface of the chitosan. In addition to its antibacterial, anti-inflammatory, and proliferative properties, chitosan can facilitate the binding of several antioxidant compounds possessed by *M. oleifera*, such as 1,3,4,5-tetrahydroxy-cyclohexane carboxylic acid and Hexadecanoic acid, both of which have antibacterial potential [43]. Resveratrol, a polyphenol found in Moringa leaves, has anti-inflammatory, antioxidant, antitumor, and immunomodulatory properties [44].

Figure 5 shows the weight of the chemical elements possessed by crab shell nano chitosan, namely oxygen (69.4%), calcium (19.7%), magnesium (6.6%), and phosphorus (4.3%). While the amount of atomic oxygen (82.8), calcium (9.4%), magnesium (5.2%), and phosphorus (2.6). Predoi reported that chitosan coated with MgHApCh contained chemical elements such as Oxygen, Calcium, Magnesium, and phosphorus with different atomic weights [45]. Still, the higher oxygen content of nano chitosan was used in this study. Reported that the quantity of oxygen content in a restorative material is higher than the function of drug delivery to increase its role at the healing target location [46]. Oxygen is also needed by cells and tissues during the healing process, increasing tissue conductivity, decreasing proinflammatory cytokine responses, and inhibiting reactive oxygen species activity that undergoes infection [47].

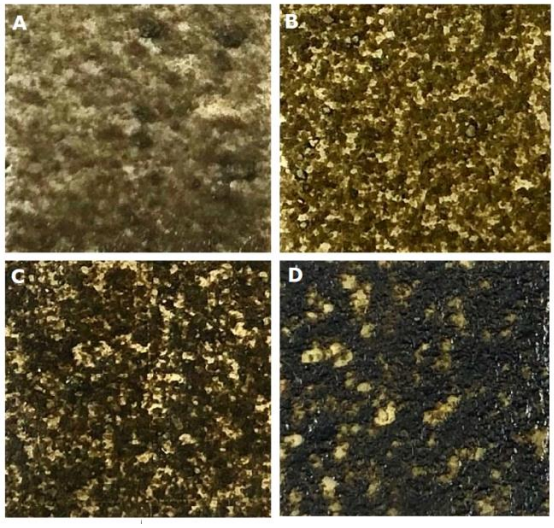


Figure 4. Surface morphology of chitosan nano-membrane films from crab shells added with Moringa leaf resveratrol. (A) chitosan nano membrane film, (B) chitosan nano membrane film + 10% resveratrol, (C) Chitosan nano membrane film + 20% resveratrol, (D) Chitosan nano membrane film + 30% resveratrol: Scale bars (200 µm).

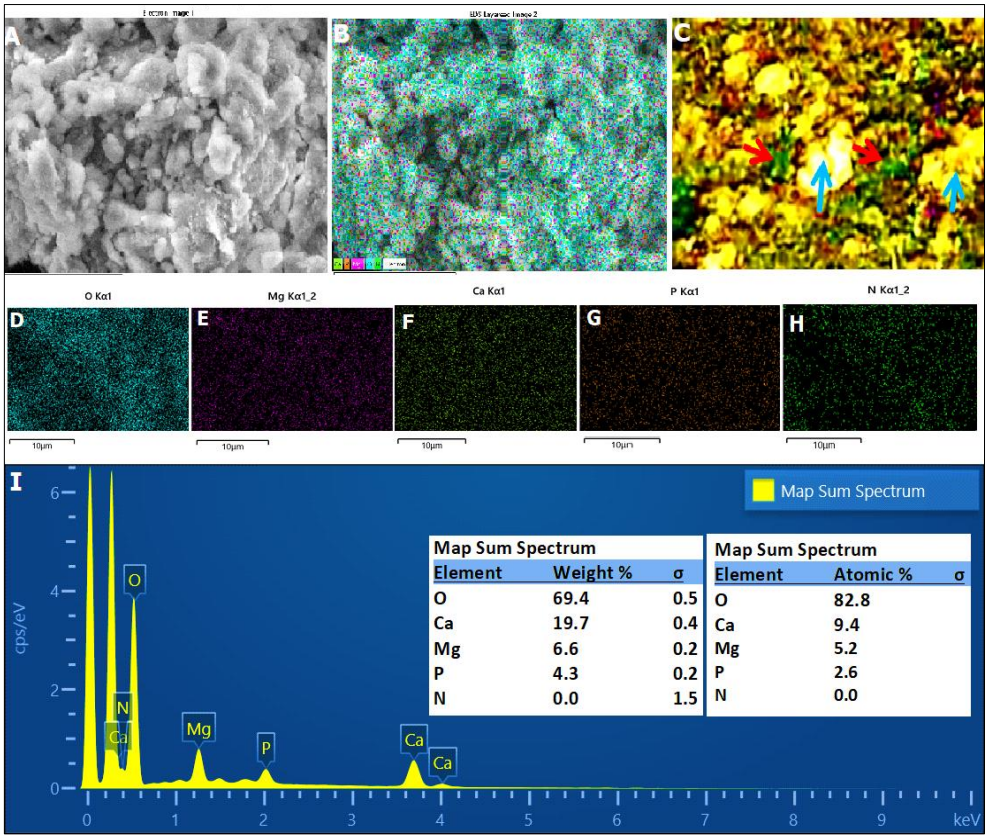


Figure 5. The SEM profile of resveratrol nano chitosan was seen with SEM-EDS at 10 m magnification. (A) Full image of nano chitosan of crab shell (B) chitosan surface detected by EDS (C) NCHR membrane film surface profile. Red arrows (active material of Moringa leaf extract) and blue arrows (Crab shell of chitosan nano). Scale bar 200 µm. The surface area of chemical elements detected from NCHR (D) Oxygen, (E) Magnesium, (F) calcium, (G) Phosphorus, (H) Nitrogen, and (I) EDS peak profile which shows the percentage of the chemical element content.

Nano chitosan from crab shells contains many chemical elements that serve as chelating agents to improve biological functions in tissue repair and increase inflammatory cell responses in wound healing, as shown in Figure 4. One of the functions of chitosan's chelating agent is to bind toxic metal ions to form complex structures that are easily removed intracellularly or extracellularly from the body [48]. In wound healing and tissue repair, chitosan also facilitates nutritional supplementation and influences the incorporation of amino acids into collagen proteins. Another function is to bind metals in the body and regulate the number of metals so that toxic metal levels decrease [49]. Heavy metals interfere with cellular activities, such as growth, proliferation, differentiation, damage repair, and apoptosis and induce toxicities such as the production of reactive oxygen species, weakened antioxidant defenses, enzyme inactivation, and oxidative stress [50].

Calcium in the film membrane of nano chitosan from crab shell chitosan is anticipated to enhance wound healing [51]. Calcium not only acts as an essential clotting factor in the early stages of the healing process but also plays an essential role as an extracellular regulator and internal modulator of keratinocyte and fibroblast processes [52]. In addition, calcium is reported to be the initial step in repairing damaged tissue, particularly as Factor IV in the hemostatic phase, which promotes migration and regeneration of epidermal cells during a later phase of healing [53, 54].

As Kawai et al. (2011) reported, calcium has a therapeutic effect on wound healing via multiple mechanisms, resulting in a more rapid and effective recovery [55]. Calcium enables T cells to utilize sugar to fight infection. Calcium signals regulate immune cells by supplying them with the nutrients necessary for enhanced cellular responses against infectious agents such as bacteria, viruses, and parasites [56]. The activity of extracellular receptors mediates this response to modulate inflammation-induced resorption [57]. Oxygen is also believed to play a significant role in each phase of the wound-healing process and to increase the host's resistance to infection.

Consequently, any interruption in the oxygen supply can retard healing [58]. Hypoxia promotes wound healing by triggering the release of growth factors and angiogenesis, whereas oxygen is required to sustain the healing process [59]. Moreover, oxygen can kill bacteria and viruses and activate proinflammatory cytokine genes to regulate immune responses to infectious agents. In addition to oxygen, magnesium has been implicated in wound healing by activating the collagenase enzyme involved in repairing injured tissue. In addition, it maintains the nerve impulse response necessary for muscle contraction and relaxation [60]. Phosphorus, possessed by chitosan, is reported to work in inducing cell chemotaxis and peptides related to the calcitonin gene that are relevant to wound healing, as well as neuropeptides such as nerve growth factor [61]. Phosphorus also functions as an antibacterial agent and is essential for the body's production of adenosine triphosphate, a molecule that stores energy. Phosphorus also facilitates the body's use of carbohydrates and fats to synthesize proteins to grow, maintain, and repair cells and tissues [62].

3-4- pH assessment of Nano Chitosan Resveratrol

Table 2 reports the pH of the film membrane of Nano chitosan before and after incubation in different solvents for 24 hours. After 24 hours, the solvent decreased in pH, but not significantly. Its reduction was influenced by the resveratrol concentration added to the chitosan film. It is the assumption that the film membrane of nano chitosan can maintain the stability of changes in pH, as reported in Figure 6, where the membrane film dissolved in PBS and Aquadest has better change stability based on the percentage of pH stability. The strength of a solution's pH is determined by the chemical elements' ionic properties and composition [63]. In addition, the flow rate of the solution also affects its properties as a solvent. The flow rate is correlated with the pH of the solution and the properties of the chemical elements contained therein [64].

Table 2. pH changes of films membrane of NCHR in various solvents before and after incubation 24 hours

Solvents	Incubation (0 hours)				Incubation (24 hours)			
	NCHR 10%	NCHR 20%	NCHR 30%	NCH	NCHR 10%	NCHR 20%	NCHR 30%	NCH
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
DMSO	9.71±0.02	9.82±0.01	9.72±0.11	9.90±0.02	9.65±0.04	9.76±0.05	9.64±0.08	9.92±0.05
NaCl 0.9%	6.72±006	6.75±0.04	6.87±0.10	7.05±0.11	6.70±002	6.73±0.01	6.64±0.03	6.88±0.01
PBS	7.01±0.00	7.02±0.00	7.05±0.00	7.08±0.06	6.88±0.05	6.80±0.08	6.83±0.06	7.04±0.15
Aquadest	6.98±0.00	6.90±0.00	6.98±0.00	6.91±0.01	6.90±0.00	674±0.02	6.81±0.04	6.87±0.02
n	12	12	12	12	12	12	12	12
*p=value	0.020	0.023	0.024	0.045	0.023	0.033	0.025	0.033
	0.348				0.171			

* Kruskal Wallis Test; NCH (Nano Chitosan); NCHR (Nano Chitosan Resveratrol); SD (Standard Deviation).

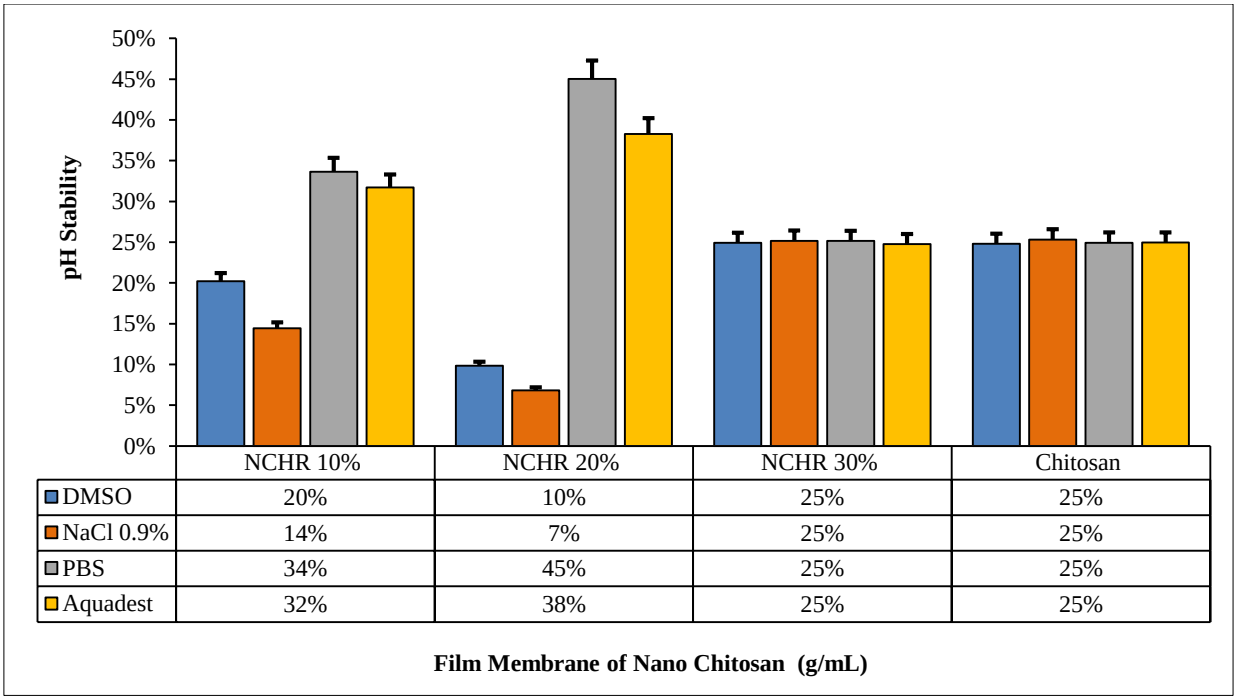


Figure 6. pH stability of film membrane in different solvents. The membrane film with 30% resveratrol and Nano chitosan had better stability of pH changes than the addition of 10% and 20% resveratrol. Bar (pH stability) and Bar error (error bar with percentage).

According to the findings of this study, the film-membrane of nano chitosan containing resveratrol has good pH stability. Applying it to the wound can indicate the film's absorption properties in cell fluid. According to Nunthanid (2001), PBS solvent and aquadest at pH 7.4 can help increase cross-linking between chitosan and phosphate anion to increase film strength [65]. Furthermore, the pH stability of the chitosan film is closely related to the properties of water vapor permeability, tensile strength, elongation, and solubility [66].

The chitosan nanofilm without resveratrol demonstrated excellent stability in this study. It means that chitosan films have good biodegradation properties in various solutions, particularly cellular and extracellular fluids, both psycho-chemically and biologically. The intracellular pH ranges physiologically from 7.0 to 7.4 [67], similar to the pH of PBS, physiological NaCl, and Aquades used in this study. Resveratrol can interact with solvents with varying pH levels. As a result, the film membrane of nano chitosan containing resveratrol may be used as drug delivery to improve wound healing by increasing keratinocyte and fibroblast proliferation in pH 7.2 to 8.3 environments [68].

In Figure 6, it was reported that the membrane film added with 30% resveratrol and the film membrane of nano chitosan without resveratrol showed excellent stability of pH changes compared to other solvents ($p<0.05;0.000$). The average strength of the pH of the two test materials for each solvent reached 25%. It means that the resveratrol mixture in the film of nano chitosan lm can help maintain the stability of changes in pH at a concentration of 30% resveratrol very well compared to 10% and 20% resveratrol. In this study, after incubating the film membrane of nano chitosan for 24 hours in various solvents, the pH decreased to slightly acidic in a neutral pH solvent. Because acidic pH has been reported to prevent the development of cancer cells [69], but it can also maintain cell and tissue repair in wound healing [70], this decrease in pH indicates that the resveratrol chitosan membrane film provides an opportunity to improve wound healing. According to Nagoba (2015), the slightly acidic pH of the liquid (solvent) promotes wound healing by controlling wound infection, altering the activity of damaging enzymes, releasing oxygen, and increasing epithelialization and angiogenesis [71].

3-5- Solubility of Nano Chitosan Resveratrol

Table 3. It was reported that DMSO solvent had very low solubility, while NaCl, PBS, and aquadest solvents increased the solubility above 95%. It means that these three solvents have a perfect solubility response in describing the structure or active compounds that make up the membrane film, so it is inevitable that this solution can be used as the solution of choice in researching membrane film applications to be applied to surgical wounds. Solubility properties are strongly influenced by the pH of each solution, as reported in Table 2. However, DMSO is often used topically to reduce pain and accelerate wound healing, burns, and muscle and bone injuries.

Table 3. Solubility assessment of film membrane of NCHR in various solvents

Nano Chitosan Resveratrol	Solvents (%)			
	DMSO	NaCl 0.9%	PBS	Aquadest
NCHR 30%	27.3	99.9	92.3	90.0
NCHR 20%	33.3	99.9	98.3	98.9
NCHR 10%	85.7	99.8	98.9	97.1
Nano Chitosan	30.8	99.2	98.6	98.9

NCHR (Nano chitosan Resveratrol), DMSO (Dimethyl sulfoxide), PBS (Phosphate Buffer Saline)

DMSO is also used topically to treat inflammation, osteoarthritis, and rheumatoid arthritis [72]. At large doses, for a long time, it shows only minor toxic effects such as mild skin irritation, itching, and burning. Although DMSO is absorbed rapidly through the skin, it has a low level of toxicity [73]. Although NaCl 0.9%, PBS, and aquadest can dissolve membrane films better, their penetration ability needs to be considered. In addition, the pH factor is also thought of as the nature of its solubility. Where pH determines the hydrophobic core of the membrane film surface, alkaline pH tends to increase the hydrophobicity of the membrane film surface. DMSO has an alkaline pH, which is one factor in its longer solubility.

3-6- Swelling Assessment of Nano Chitosan Resveratrol

Table 4 shows that NaCl 0.9%, PBS, and aquadest solvents have excellent swelling indices. In contrast, DMSO solvent has a swelling index above 60%-90%. In general, all solvents in this test have different values. This difference is determined by the chemical properties of each solvent and the biological response to the film degradation process, as shown in Figure 7. Swelling polymers from nano chitosan increases mechanical bonding by opening mucoadhesive sites for hydrogen bonding or electrostatic interactions between polymers and tissues. This concept is essential to be applied to surgical wounds [74].

Table 4. Swelling assessment of film membrane of NCHR

Nano Chitosan Resveratrol	Solvents (%)			
	DMSO	NaCl 0.9%	PBS	Aquadest
NCHR 30%	62	92	100	92
NCHR 20%	67	90	100	100
NCHR 10%	70	100	100	100
Nano Chitosan	90	100	100	93

NCHR (Nano chitosan Resveratrol), DMSO (Dimethyl sulfoxide), PBS (Phosphate Buffer Saline)

The swelling characteristics of chitosan films were employed to test the wound-healing process's solubility and reactivity to intracellular and extracellular fluids [75]. The swelling film was described as a procedure for the flow of antibacterial and antioxidant-functioning active chemicals. In addition to serving in the drug delivery process, a good swallowing film extends the absorption phase of the active material in the wound area, aiding the immune system or promoting cell proliferation and tissue repair [76]. Good swelling depends on the reaction to bodily fluids and the adaptation of the wound environment. It depends on the nature of pH and temperature, as these two factors influence the film swelling process. Chitosan film has good swelling properties, which correlate with decreased allergic response [77].

The swelling behavior of chitosan hydrogel depends on the amount and type of solvent and its ionic content. When the amount of water in the surrounding liquid increases, the difference in ionic concentration also increases, resulting in more significant swelling due to higher osmotic pressure [78]. Our findings demonstrated that PBS, Aquadest, and 0.9% NaCl solvents have greater swelling properties than DMSO for chitosan films. This difference is directly related to the pH of the solution, as DMSO has an alkaline pH, whereas aquadest solvents, 0.9% NaCl, and PBS, have a neutral pH (Table 2). The swelling process of the film in all buffer solutions was related to kinetic properties, according to experimental results. The swelling in acidic and neutral solutions is identical. In an alkaline solution, however, the enlargement of the film rises [79].

3-7- Permeability Assessment of Nano Chitosan Resveratrol

Table 5 shows that the film membrane of NCHR has an excellent WVTR index in all solvents. The average value of R^2 for each solvent was above 0.95 except for the PBS group with the film membrane of 30%NCHR (R^2= 0.85), and the equates group at 20% NCHR (R^2=0.84) and the nano chitosan group (R^2=0, 88). However, this value is still a firm tolerance limit. All groups of solvents used in this study have excellent water vapor-lowering ability, so they are not

easily damaged. The WVTR value measures the water vapor that passes through the membrane film. The WVTR value is determined based on the value of a straight-line range (standard curve) [18]. Oxygen transmission rate (OTR) and vapor transmission rate (WVTR) are the two main ingredient specification properties that determine the shelf life of packaging [80].

Table 5. WVTR assessment of film membrane of NCHR influenced by various solvents

Film membrane of Nano Chitosan Resveratrol	DMSO Solvent (g/m² × 24 h)				
	n	Mean±Sdev	WVTR Value (%)	Standard Curve	
				y	R²
NCHR 30%	6	0.0854±0.048	9%	0.0294x - 0.0023	0.981
NCHR 20%	6	0.0838±0.049	8%	0.0296x - 0.0042	0.986
NCHR 10%	6	0.0932±0.050	9%	0.0315x - 0.0012	0.988
Nano Chitosan	6	0.0838±0.049	8%	0.0295x - 0.004	0.985
Film membrane of Nano Chitosan Resveratrol	**NaCl 0.9% Solvent (g/m² × 24 h)**				
	n	Mean±Sdev	WVTR Value (%)	Standard Curve	
				y	R²
NCHR 30%	6	0.046±0.0157	4%	0.0122x + 0.0079	0.941
NCHR 20%	6	0.118±0.049	12%	0.0337x + 0.0142	0.930
NCHR 10%	6	0.1106±0.085	11%	0.0459x - 0.0226	0.938
Nano Chitosan	6	0.114±0.037	11%	0.0299x + 0.0203	0.947
Film membrane of Nano Chitosan Resveratrol	**PBS Solvent (g/m² × 24 h)**				
	n	Mean±Sdev	WVTR Value (%)	Standard Curve	
				y	R²
NCHR 30%	6	0.059±0.023	6%	0.0159x + 0.0094	0.853
NCHR 20%	6	0.061±0.041	6%	0.0235x - 0.0078	0.977
NCHR 10%	6	0.107±0.045	11%	0.0314x + 0.0109	0.957
Nano Chitosan	6	0.066±0.040	7%	0.0239x - 0.0045	0.978
Film membrane of Nano Chitosan Resveratrol	**Aquadest Solvent (g/m² × 24 h)**				
	n	Mean±Sdev	WVTR Value (%)	Standard Curve	
				y	R²
NCHR 30%	6	0.070±0.036	7%	0.0228x + 0.0018	0.965
NCHR 20%	6	0.031±0.028	3%	0.014x - 0.0085	0.843
NCHR 10%	6	0.023±0.0071	2%	0.0058x + 0.0048	0.910
Nano Chitosan	6	0.025±0.0140	3%	0.0083x + 0.0009	0.888

NCHR (Nano chitosan Resveratrol), DMSO (Dimethyl sulfoxide), PBS (Phosphate Buffer Saline), NaCl (Natrium Chloride).

The WVTR index was utilized to evaluate the efficacy of preparing porous chitosan-coated cellulose composite membranes for prospective usage as wound dressings [81]. The permeability value can be used as a measure to describe the ability of the NCHR membrane film to sustain moisture permeability outside of the wound application environment. WVTR is the continuous flow of water vapor in unit time across a unit area of matter, normal to specified parallel surfaces, under specific temperature and humidity parameters on each character [82].

In this study, the permeability of membrane films was measured using four solvents. The 0.9% NaCl solvent has the most excellent effect on enhancing the permeability films membrane of NCHR (average of 10%), followed by DMSO solvents (average of 9%), PBS (average of 7%), and Aquadest (average of 4%). When the membrane film responds to a wound environment impacted by intracellular and extracellular fluids, these four solvents are used as a simulation. 0.9% NaCl solution is a crystalloid fluid that contributes to the body's fluid and electrolyte balance.

Simulations in vitro demonstrated that 0.9% NaCl had a more significant impact on the permeability changes of NCHR membrane films. This assay delivers valuable information when conducting tests on experimental animals using 0.9% NaCl analytical solutions. NaCl 0.9% is used to restore lost body fluids and salts, dilute other drugs and act as a sterile fluid to wash wounds, nasal passages, or surgical sites. The variation in the WVTR index, which was influenced by the solvent type, may be due to variances in chitosan film morphology generated by the interaction of Carboxymethyl *cellulose* (CMS) and glycerol with various chitosans, glycerol being more polar than CMS [83]. Due to intermolecular interactions between the chitosan polymer and the active ingredient of resveratrol, Chitosan-CMS was capable of producing partial cross-links with chitosan at higher resveratrol concentrations [84].

The WVTR index of NCHR membrane film impacted by 0.9% NaCl was superior to that of other solvents. It can be assumed that permeability calculation is independent of layer thickness. This assumption does not apply to edible films that are hydrophilic. The maximum permeability value of the NCHR membrane film in this investigation, which was affected by a 0.9% NaCl solution, indicates that this solution will increase the complexity of the water transport mechanism to and from the hydrophilic chitosan film [85]. The nonlinear water absorption isotherm and the water content-dependent diffusion isotherm contribute to this complexity [86]. When the film is cationic and contains hydrophilic water molecules, it interacts with the polymer matrix and enhances water vapor permeability, according to a report. The rise in water vapor corresponds to the increase in humidity, which causes the film to inflate [87]. The swelling creates a conformational shift in the film's architecture, which increases its water absorption. In addition, raising the membrane film's permeability results in channels inside the polymer structure, which can facilitate an increase in permeate flow [88]. As a result of swelling, the structure of the polymer has been altered to respond to the tension in the chitosan film during the absorption process [89].

3-8- Biodegradation Evaluation of Nano Chitosan Resveratrol

Table 6 reports the biodegradation of NCHR membrane films in different solvents, where the DMSO group showed lower biodegradation properties, as reported in Figures 7 and 8. The assessment of the average value of 1-10 minutes of biodegradation treatment time showed that the aquadest solvent was able to higher film degradation (39%) than 0.9% NaCl and 29% PBS respectively. Meanwhile, the DMSO solvent had a lower film-dissolving ability (3%) (Figure 7). This biodegradation property can be used as a reference for formulating a mixture of chitosan films applied to surgical wounds with different properties and healing times, such as bone, tissue, muscle, and mucosa.

Table 6. Biodegradation of film membrane of NCHR

Time (Minutes)	n	DMSO -Biodegradation (600 nm)			
		NCHR 30%	NCHR 20%	NCHR 10%	Nano Chitosan
		Mean±Sdev	Mean±Sdev	Mean±Sdev	Mean±Sdev
1	3	0.048±0.000	0.046±0.000	0.046±0.000	0.048±0.001
2	3	0.058±0.001	0.049±0.002	0.048±0.005	0.046±0.001
4	3	0.069±0.001	0.054±0.001	0.047±0.005	0.047±0.001
6	3	0.071±0.003	0.052±0.001	0.048±0.060	0.049±0.005
8	3	0.071±0.001	0.052±0.001	0.048±0.001	0.047±0.005
10	3	0.073±0.005	0.054±0.005	0.047±0.001	0.046±0.005
*p-value	18	0.041	0.012	0.043	0.002
Time (Minutes)	n	NaCl 0.9%-Biodegradation (600 nm)			
		NCHR 30%	NCHR 20%	NCHR 10%	Nano Chitosan
		Mean±SD	Mean±SD	Mean±SD	Mean±SD
1	3	0.521±0.037	0.688±0.142	0.954±0.303	0.352±0.202
2	3	0.494±0.090	0.434±0.055	0.0492±0.023	0.154±0.067
4	3	0.889±0.024	0.666±0.097	0.795±0.134	0.187±0.025
6	3	0.401±0.095	0.546±0.045	1.132±0.079	0.218±0.032
8	3	0.323±0.127	0.543±0.027	1.054±0.106	0.303±0.127
10	3	0.309±0.040	0.545±0.093	0.855±0.097	0.128±0.001
*p-value	18	0.003	0.022	0.047	0.001
Time (Minutes)	n	PBS Biodegradation (600 nm)			
		NCHR 30%	NCHR 20%	NCHR 10%	Nano Chitosan
		Mean±SD	Mean±SD	Mean±SD	Mean±SD
1	3	0.581±0.170	0.444±0.136	0.84±0.357	0.217±0.111
2	3	0.691±0.1998	0.44±0.087	0.709±0.221	0.23±0.067
4	3	0.672±0.078	0.463±0.154	0.634±0.153	0.182±0.019
6	3	0.538±0.104	0.585±0.165	0.945±0.304	0.152±0.044
8	3	0.559±0.245	0.473±0.114	0.851±0.209	0.184±0.014
10	3	0.701±0.279	1.045±0.252	0.738±0.147	0.205±0.001
*p-value	18	0.004	0.05	0.021	0.003

Time (Minutes)	n	Aquadest Biodegradation (600 nm)			
		NCHR 30%	NCHR 20%	NCHR 10%	Nano Chitosan
		Mean±SD	Mean±SD	Mean±SD	Mean±SD
1	3	1.017±0.070	0.68±0.040	0.723±0.078	0.305±0.076
2	3	0.782±0.1211	0.685±0.104	0.735±0.251	0.221±0.015
4	3	1.063±0.133	0.893±0.185	0.761±0.037	0.228±0.0671
6	3	1.266±0.127	1.009±0.073	0.792±0.057	0.241±0.024
8	3	1.219±0.123	1.099±0.176	0.567±0.069	0.211±0.008
10	3	1.29±0.189	0.833±0.155	0.516±0.049	0.189±0.002
*p-value	18	0.004	0.05	0.021	0.003

* One Way Anova, NCHR (Nano Chitosan Resveratrol), DMSO (Dimethyl sulfoxide), PBS (Phosphate Buffer Saline), NaCl (Natrium Chloride)

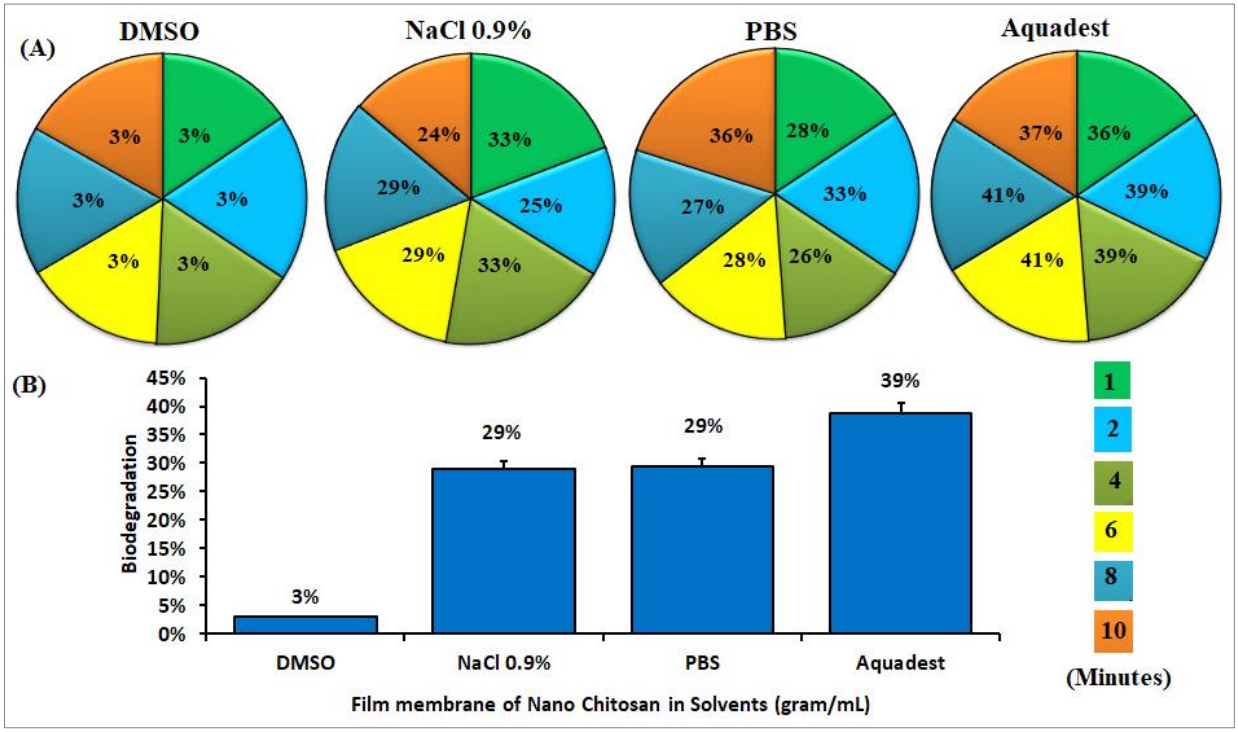


Figure 7. Biodegradation of film membrane of NCHR with different solvents(A) Biodegradation of film membrane of nano chitosan in various solvents based on treatment time (minutes). (B) Percentage of total biodegradation of film –membrane of nano chitosan various solvents. The aquadest solvent has higher biodegradation properties than other solvents (NaCl 0.9%, PBS, and DMSO).

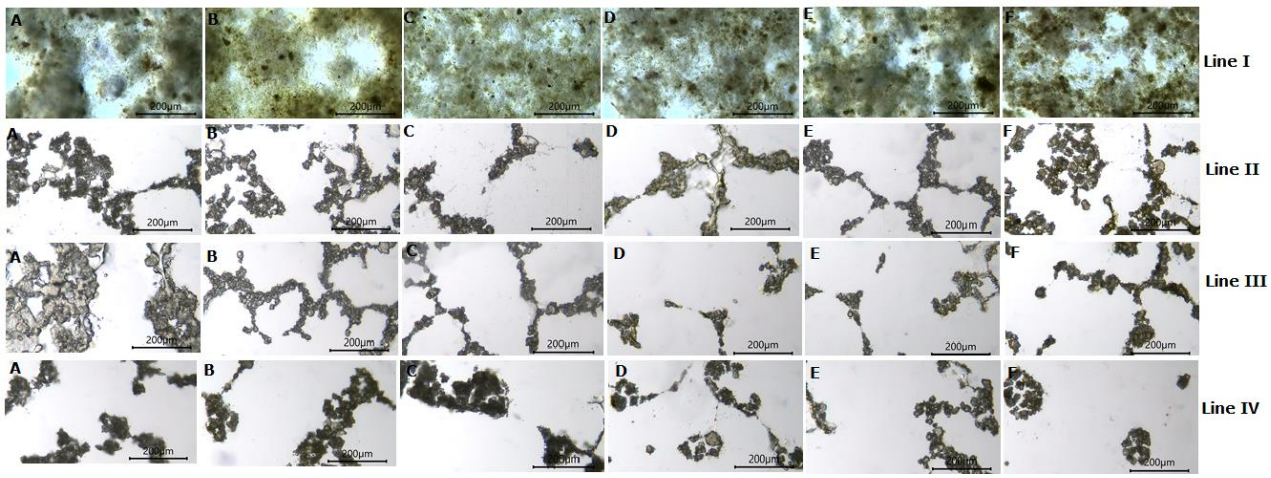


Figure 8. Biodegradation of film membrane of NCHR with different solvents. Line 1 (DMSO), Line 2 (Nacl 0.9%), Line 3 (PBS), and Line 4 (Aquadest). Each solvent used a different time to measure the degradation of the film (A) 1 minute, (B) 2 minutes, (C) 4 minutes, (D) 6 minutes, (E) 8 minutes, (F) 10 minutes. Bar scale 200 µm.

In the DMSO solvent group, the degradation of the film for 1, 2, 4, 6, 8, and 10 minutes exhibited the same solubility (3%), as shown in Figure 6. In the 0.9% NaCl group at 1 and 4 minutes, film degradation was more significant (33%), while in the PBS group at 10 minutes, film degradation was better (36%), and in the solvent group with distilled water, film degradation was greater (41%). Figure 7 depicts the qualitative data as a representation of Figure 6's depiction of the degradation of the resveratrol chitosan film as a result of exposure to various solvents.

According to the data, 0.9% NaCl and PBS exhibited superior biodegradability compared to DMSO and aquadest. The degradation time of plant-derived active substances used for wound dressing must coincide with the healing phase [90]. Over time, the dissolution of the active substance from a film, capsule, or membrane sheet is greatly anticipated. According to reports, the size of the solution's pH significantly affects its solubility because the pH balance (neutral pH) influences the solution's ionic and catalytic properties [91]. NaCl 0.9% and PBS possess ionic characteristics and steady conductivity, enhancing the materials' conductivity transfer [92]. The conductivity qualities follow the change of the material mixed with the solution, which is consistent with the time-dependent degradation properties.

The biodegradation value can be used as a reference for use in the film mechanization process before being applied to post-surgical wounds. These solvents have different biomechanical properties when degrading the chitosan film structure [93]. Hopefully, this examination can serve as a reference for the application of films to surgical sites or sites, both in bone surgery and surgical wounds on tissue or mucosa. The biodegradation of films as drug delivery is primarily determined by pH, temperature, and the composition of the constituent elements of the film [94]. The film membrane of NCHR with an alkaline pH has a lower solubility response in the chitosan film experiment. The tendency for structural changes and hydrogen bonds is higher at an acidic or neutral pH, causing the film to break more easily and dissolve [95]. Biodegradation testing is determined using films for drug delivery at surgical sites and locations. The degradation of the chitosan film or other drug bases is expected to be in line with the healing time [96].

Chitosan is regarded as a highly biocompatible biopolymer due to its biodegradability, bioadhesives, and bioactivity, allowing it to be used in a variety of applications. As a wound dressing, chitosan as a film preparation is anticipated to disintegrate. The increase in film breakdown is expected to boost wound absorption of active chemicals. Not only is the disintegration of chitosan film affected by the entry of cellular and extracellular fluids, but also by the enzymatic response to become non-toxic components. Chitosan can be digested in vivo by the nonspecific enzyme lysozyme, which is present in all mammalian tissues. This enzyme has a pH of 5.2, and its activity is influenced by temperature and pH levels [97].

3-9-Antibacterial Activities of Nano Chitosan Resveratrol

Tables 7 and 8 report oral bacteria's inhibition (*Porphyromonas gingivalis*), the film membrane of NCHR with various concentrations could maintain bacterial cell populations (assessed by spectrophotometry) below 300 CFU/mL. The bacteriostatic properties of the membrane films are in line with Table 8, where the MIC membrane film test showed a solid inhibitory scale (> 15 mm) at 24 hours and 48 hours. Except for 24 hours of the chitosan nano group with the medium shell. However, the NCHR, with various concentrations, can control the growth of bacteria. Another finding is that the MIC properties of the membrane film in Table 8 decrease but are still in the sensitive range. Based on the spectrophotometric assessment, the reference value for the growth of S. mutans is OD 0.05 nm (<150 CFU/mL), 0.08–0.1 nm (Mc Farlan 0,5; <300 CFU), OD 0,11-0,29 nm (Mc Farland 1; 300–600 CFU); OD 0,3-0,49 nm (Mc Farland 2; 600–1200 CFU. Mc. Farland Standard for in vitro use only, Catalogue No. TM50-TM60, Scott Sutton, Measurement of Microbial Cells by Optical Density, 2011) [24].

Table 7. Growth of *Porphyromonas gingivalis* under the influence of film membrane of NCHR with different solvents

Nano Chitosan Resveratrol	n	*Porphyromonas gingivalis* Growth (600 nm)							
		DMSO		NaCl 0.9%		PBS		Aquadest	
		Mean±Sdev	Colony (CFU/mL)	Mean±SD	Colony (CFU/mL)	Mean±SD	Colony (CFU/mL)	Mean±SD	Colony (CFU/mL)
NCHR 30%	3	0.065±0.043	<300	0.078±0.009	<300	0.071±0.008	<300	0.086±0.011	≤300
NCHR 20%	3	0.024±0.001	<200	0.068±0.017	<300	0.058±0.006	<300	0.062±0.015	<300
NCHR 10%	3	0.077±0.016	<300	0.055±0.009	<300	0.057±0.010	<300	0.046±0.002	<300
Nano Chitosan	3	0.066±0.027	<300	0.066±0.007	<300	0.046±0.003	<200	0.072±0.021	<300
*p-value	12	0.005		0.049		0.001		0.000	

* One Way ANOVA; NCHR (Nano Chitosan Resveratrol), DMSO (Dimethyl sulfoxide), PBS (Phosphate Buffer Saline), NaCl (Natrium Chloride)

Table 8. Minimal Inhibition Concentration of *Porphyromonas gingivalis* in the effect of film membrane of NCHR based on incubation time

Nano Chitosan Resveratrol	n	Minimal Inhibition Concentration (mm)						*p-value
		24 hours			48 hours			
		Mean±Sdev	Sensitive	Frequency	Mean±Sdev	Sensitive	Frequency	
NCHR 30%	5	16.02±1.15	Strong	24%	16.6±2.05	Strong	27%	0.077
NCHR 20%	5	18.93±2.54	Strong	29%	15.54±1.03	Strong	25%	
NCHR 10%	5	16.56±1.37	Strong	25%	15.05±0.21	Strong	24%	
Nano Chitosan	5	14.1±0.27	Medium	21%	15.04±1.02	Strong	24%	
* p=Value	20		0.031			0.062		

* One Way ANOVA; NCHR (Nano Chitosan Resveratrol), DMSO (Dimethyl sulfoxide), PBS (Phosphate Buffer Saline), NaCl (Natrium Chloride)

Bhargav (2016) reported that the inhibition zones at values of 100 µg/disc and the minimum inhibitory concentrations (MIC) for the four bacterial strains were in the range of 11.0–20.0 mm and 125–250 g/mL, respectively [98]. Generally, standard antibiotics for ampicillin with a range of < 11 mm are insensitive, 12–13 mm are quite sensitive, and > 13 mm are very sensitive. The film membrane of NCRH tested in this study is very sensitive to bacterial growth. Dai (2011) reported that chitosan could be used to prevent or treat wound and burn infections because it has antimicrobial properties and can also act as drug delivery to increase growth factors in wound healing [99]. Chitosan can prevent or treat wound and burn infections due to its inherent antimicrobial properties and ability to deliver extrinsic antimicrobial agents to burns. It can also be used as a slow-release drug delivery vehicle for wound-healing growth factors [100].

Due to its inherent antibacterial properties, chitosan inhibits the growth of bacteria and fungi, such as Staphylococcus aureus. The application of chitosan as an antibacterial disrupts the structural membrane wall, thereby increasing the damage to cell structures on the surface of the cell wall and the death of bacteria due to the failure of active transport between the extracellular and intracellular compartments [101]. Chitosan possesses antimicrobial properties that inhibit the ROS system of pathogenic cells. In conjunction with natural resveratrol, bacterial oxidation is inhibited by increasing wound healing growth factors [99]. The ability of resveratrol-labeled chitosan to act as a bacterium in vitro is a reference for its use in preventing an increase in infections and accelerating wound healing [102]. Theoretically, Yilmaz Atay (2019) describes the mechanism of action of chitosan as an antibacterial as penetrating negatively charged bacterial cell walls, causing interference with cell interactions with the surrounding environment, thereby altering membrane permeability and toxicity to DNA, which inhibits DNA replication and causes cell death [103].

4 - Conclusion

The nano chitosan of crab shell includes the Ethane chemical 1,1-diethoxy- (CAS) 1,1-Diethoxye (81.06%) as the antitoxic and anti-inflammatory. Additionally, it contains the active compounds of 3-penten-2-one 4-methyl and Terpene d-Limonene, which function as anti-inflammatory, immunomodulatory, antiproliferative, and antioxidant agents. The chemical components present in crab shell micro chitosan are oxygen (82.8%), calcium (9.4%), magnesium (5.2%), and phosphorus (2.8%).

The membrane film of 30% NCHR and the film membrane of nano chitosan had excellent stability of pH changes (25% each) in all solvents (DMSO, 0.9% NaCl, PBS, and aquadest). In addition, 0.9% NaCl solvent has better solubility properties against the film membrane of NCHR than all other solvents (99% each). Swelling of the film membrane of NCHR was very good in PBS and 0.9% NaCl solvents, and the best changes in the permeability of the film membrane of NCHR were NaCl 0.9% (average of 10%), DMSO solvents (average of 9%), PBS (average of 7%), and aquadest solvents (average of 4%). NaCl 0.9% and PBS have better biodegradation properties against the film membrane of NCHR than the solvents DMSO and aquadest. All solvents with varying concentrations of each film membrane of NCHR and the film membrane of nano chitosan film without resveratrol had strong bacteria inhibitory effects for 24 and 48 hours.

5 - Nomenclatures

DMSO	Dimethyl Sulfoxide	NaCl	Natrium Chloride
PBS	Phosphate Buffer Saline	WVTR	Water Vapor Transmission Rate
OTR	Oxygen Transmission Rate	NCHR	Nano Chitosan Resveratrol
NCH	Nano Chitosan	CMC	Carboxymethyl Cellulose
SEM-EDS	Scanning Electron Microscope-Energy Dispersive X-Ray		

6- Declarations

6-1- Author Contributions

Conceptualization, B.A.G., and N.A.; methodology, B.A.G., and D.S., and A.I.N.; software, S.R., and M.N.; validation, B.A.G., S.J., M.N., N.S., and C.S.; writing—original draft preparation, M.N., S.J., D.S.; C.S., and S.R.; writing—review and editing, B.A.G., and S.J.; visualization, N.S.; supervision, D.S., SRJ., and C.S.; project administration, B.A.G., and S.J.; funding acquisition, B.A.G. All authors have read and agreed to the published version of the manuscript.

6-2- Data Availability Statement

The data presented in this study are available in the article.

6-3- Funding

Directorate of Research, Technology, and Community Service Directorate General of Higher Education, Research, and Technology, Ministry of Education, Culture, Research, and Technology, Indonesia. Fund Number: 145/E5/PG.02.00.PT/2022.

6-4- Acknowledgements

The authors acknowledged the Ministry of Education, Culture, Research, and Technology of Indonesia for assisting this research.

6-5- Institutional Review Board Statement

Not applicable.

6-6- Informed Consent Statement

Not applicable.

6-7- Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

7- References

[1] Baker, S. B., Xiang, W., & Atkinson, I. (2017). Internet of Things for Smart Healthcare: Technologies, Challenges, and Opportunities. IEEE Access, 5, 26521–26544. doi:10.1109/ACCESS.2017.2775180.

[2] Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., & Supuran, C. T. (2021). Natural products in drug discovery: advances and opportunities. Nature Reviews Drug Discovery, 20(3), 200–216. doi:10.1038/s41573-020-00114-z.

[3] Jha, R., Singh, A., Sharma, P. K., & Fuloria, N. K. (2020). Smart carbon nanotubes for drug delivery system: A comprehensive study. Journal of Drug Delivery Science and Technology, 58, 101811. doi:10.1016/j.jddst.2020.101811.

[4] Yandri, Y., Ropingi, H., Suhartati, T., Hendri, J., Irawan, B., & Hadi, S. (2022). The Effect of Zeolite/Chitosan Hybrid Matrix for Thermal-stabilization Enhancement on the Immobilization of Aspergillus fumigatus α-Amylase. Emerging Science Journal, 6(3), 505–518. doi:10.28991/ESJ-2022-06-03-06.

[5] Khan, M. I. H., An, X., Dai, L., Li, H., Khan, A., & Ni, Y. (2018). Chitosan-based Polymer Matrix for Pharmaceutical Excipients and Drug Delivery. Current Medicinal Chemistry, 26(14), 2502–2513. doi:10.2174/0929867325666180927100817.

[6] Kumar, R., & Sharma, M. (2018). Herbal nanomedicine interactions to enhance pharmacokinetics, pharmacodynamics, and therapeutic index for better bioavailability and biocompatibility of herbal formulations. Journal of Materials NanoScience, 5(1), 35-60.

[7] Arévalo-Híjar, L., Aguilar-Luis, M. Á., Caballero-García, S., Gonzáles-Soto, N., & Del Valle-Mendoza, J. (2018). Antibacterial and cytotoxic effects of Moringa oleifera (Moringa) and Azadirachta indica (Neem) methanolic extracts against strains of Enterococcus faecalis. International Journal of Dentistry, 2018. doi:10.1155/2018/1071676.

[8] Lorevice, M. V., Otoni, C. G., de Moura, M. R., & Mattoso, L. H. C. (2016). Chitosan nanoparticles on the improvement of thermal, barrier, and mechanical properties of high- and low-methyl pectin films. Food Hydrocolloids, 52, 732–740. doi:10.1016/j.foodhyd.2015.08.003.

[9] Tongdeesoontorn, W., Mauer, L. J., Wongruong, S., Sriburi, P., & Rachtanapun, P. (2011). Effect of carboxymethyl cellulose concentration on physical properties of biodegradable cassava starch-based films. Chemistry Central Journal, 5(1), 6. doi:10.1186/1752-153X-5-6.

[10] Prasathkumar, M., & Sadhasivam, S. (2021). Chitosan/Hyaluronic acid/Alginate and an assorted polymers loaded with honey, plant, and marine compounds for progressive wound healing—Know-how. International Journal of Biological Macromolecules, 186, 656–685. doi:10.1016/j.ijbiomac.2021.07.067.

[11] Comino-Sanz, I. M., López-Franco, M. D., Castro, B., & Pancorbo-Hidalgo, P. L. (2021). The role of antioxidants on wound healing: A review of the current evidence. Journal of Clinical Medicine, 10(16), 3558. doi:10.3390/jcm10163558.

[12] Jacob, S., Nair, A. B., Boddu, S. H. S., Gorain, B., Sreeharsha, N., & Shah, J. (2021). An updated overview of the emerging role of patch and film-based buccal delivery systems. Pharmaceutics, 13(8). doi:10.3390/pharmaceutics13081206.

[13] Shehabeldine, A. M., Salem, S. S., Ali, O. M., Abd-Elsalam, K. A., Elkady, F. M., & Hashem, A. H. (2022). Multifunctional Silver Nanoparticles Based on Chitosan: Antibacterial, Antibiofilm, Antifungal, Antioxidant, and Wound-Healing Activities. Journal of Fungi, 8(6), 612. doi:10.3390/jof8060612.

[14] Xiong Chang, X., Mujawar Mubarak, N., Ali Mazari, S., Sattar Jatoi, A., Ahmad, A., Khalid, M., Walvekar, R., Abdullah, E. C., Karri, R. R., Siddiqui, M. T. H., & Nizamuddin, S. (2021). A review on the properties and applications of chitosan, cellulose and deep eutectic solvent in green chemistry. Journal of Industrial and Engineering Chemistry, 104, 362–380. doi:10.1016/j.jiec.2021.08.033.

[15] Ndhlala, A., Mulaudzi, R., Ncube, B., Abdelgadir, H., du Plooy, C., & Van Staden, J. (2014). Antioxidant, Antimicrobial and Phytochemical Variations in Thirteen Moringa oleifera Lam. Cultivars. Molecules, 19(7), 10480–10494. doi:10.3390/molecules190710480.

[16] Ervolino, E., Statkievicz, C., Toro, L. F., de Mello-Neto, J. M., Cavazana, T. P., Issa, J. P. M., Dornelles, R. C. M., de Almeida, J. M., Nagata, M. J. H., Okamoto, R., Casatti, C. A., Garcia, V. G., & Theodoro, L. H. (2019). Antimicrobial photodynamic therapy improves the alveolar repair process and prevents the occurrence of osteonecrosis of the jaws after tooth extraction in senile rats treated with zoledronate. Bone, 120, 101–113. doi:10.1016/j.bone.2018.10.014.

[17] Soraya, C., M. Alibasyah, Z., Nazar, M., & A. Gani, B. (2022). Chemical Constituents of Moringa oleifera Leaves of Ethanol Extract and its Cytotoxicity against Enterococcus faecalis of Root Canal Isolate. Research Journal of Pharmacy and Technology, 3523–3530. doi:10.52711/0974-360x.2022.00591.

[18] Hasan, M., Rusman, R., Khaldun, I., Ardana, L., Mudatsir, M., & Fansuri, H. (2020). Active edible sugar palm starch-chitosan films carrying extra virgin olive oil: Barrier, thermo-mechanical, antioxidant, and antimicrobial properties. International Journal of Biological Macromolecules, 163, 766–775. doi:10.1016/j.ijbiomac.2020.07.076.

[19] de Queiroz Antonino, R., Lia Fook, B., de Oliveira Lima, V., de Farias Rached, R., Lima, E., da Silva Lima, R., Peniche Covas, C., & Lia Fook, M. (2017). Preparation and Characterization of Chitosan Obtained from Shells of Shrimp (Litopenaeus vannamei Boone). Marine Drugs, 15(5), 141. doi:10.3390/md15050141.

[20] Soraya, C., Alibasyah, Z. M., & Gani, B. A. (2022). Biomass index and viscosity values of Moringa oleifera that influenced by Enterococcus faecalis. Journal of Syiah Kuala Dentistry Society, 6(1), 1–5. doi:10.24815/jds.v6i1.21885.

[21] Yusuf, H., Husna, F., & Gani, B. A. (2021). The chemical composition of the ethanolic extract from Chromolaena odorata leaves correlates with the cytotoxicity exhibited against colorectal and breast cancer cell lines. Journal of Pharmacy & Pharmacognosy Research, 9(3), 344–356. doi:10.56499/jppres20.969_9.3.344.

[22] Rouhani, A., Ghoddusi, J., Naghavi, N., & Al-Lawati, G. (2013). Scanning electron microscopic evaluation of dentinal tubule penetration of Epiphany in severely curved root canals. European Journal of Dentistry, 7(4), 423–428. doi:10.4103/1305-7456.120673.

[23] Alvarez-Ordóñez, A., Mouwen, D. J. M., López, M., & Prieto, M. (2011). Fourier transform infrared spectroscopy as a tool to characterize molecular composition and stress response in foodborne pathogenic bacteria. Journal of Microbiological Methods, 84(3), 369–378. doi:10.1016/j.mimet.2011.01.009.

[24] Sutton, S. (2011). Measurement of microbial cells by optical density. Journal of Validation technology, 17(1), 46-49.

[25] Syafriza, D., Sutadi, H., Primasari, A., & Siregar, Y. (2020). Spectrophotometric analysis of streptococcus mutans growth and biofilm formation in Saliva and histatin-5 relate to pH and viscosity. Brazilian Research in Pediatric Dentistry and Integrated Clinic, 21, 1–11. doi:10.1590/pboci.2021.004.

[26] Rosyada, A., Sunarharum, W. B., & Waziiroh, E. (2019). Characterization of chitosan nanoparticles as an edible coating material. IOP Conference Series: Earth and Environmental Science, 230, 012043. doi:10.1088/1755-1315/230/1/012043.

[27] Jia-hui, Y., Yu-min, D., & Hua, Z. (1999). Blend films of chitosan-gelatin. Wuhan University Journal of Natural Sciences, 4(4), 476–476. doi:10.1007/bf02832288.

[28] Silverstein, R., Webster, F., & Kiemle, D., (2005). Proton NMR spectrometry. Spectrometric Identification of Organic Compounds, 7th ed.; John Wiley & Sons, New York, United States.

[29] Sorrentino, A., Gorrasi, G., & Vittoria, V. (2007). Potential perspectives of bio-nanocomposites for food packaging applications. Trends in Food Science and Technology, 18(2), 84–95. doi:10.1016/j.tifs.2006.09.004.

[30] Gouda, M., Elayaan, U., & Youssef, M. M. (2014). Synthesis and Biological Activity of Drug Delivery System Based on Chitosan Nanocapsules. Advances in Nanoparticles, 03(04), 148–158. doi:10.4236/anp.2014.34019.

[31] Ayele, T. T., Regasa, M. B., & Delesa, D. A. (2016). Evaluation of Antimicrobial Activity of Some Traditional Medicinal Plants and Herbs from Nekemte District against Wound Causing Bacterial Pathogens. Science, Technology and Arts Research Journal, 4(2), 199. doi:10.4314/star.v4i2.24.

[32] Turcant, A., Deguigne, M., Ferec, S., Bruneau, C., Leborgne, I., Lelievre, B., Gegu, C., Jegou, F., Abbara, C., Le Roux, G., & Boels, D. (2017). A 6-year review of new psychoactive substances at the Centre antipoison Grand-Ouest d'Angers: Clinical and biological data. Toxicologie Analytique et Clinique, 29(1), 18–33. doi:10.1016/j.toxac.2016.12.001.

[33] Singh, P., Shukla, R., Prakash, B., Kumar, A., Singh, S., Mishra, P. K., & Dubey, N. K. (2010). Chemical profile, antifungal, antiaflatoxigenic and antioxidant activity of Citrus maxima Burm. and Citrus sinensis (L.) Osbeck essential oils and their cyclic monoterpene, DL-limonene. Food and Chemical Toxicology, 48(6), 1734–1740. doi:10.1016/j.fct.2010.04.001.

[34] D'Alessio, P. A., Ostan, R., Bisson, J. F., Schulzke, J. D., Ursini, M. V., & Béné, M. C. (2013). Oral administration of d-Limonene controls inflammation in rat colitis and displays anti-inflammatory properties as diet supplementation in humans. Life Sciences, 92(24–26), 1151–1156. doi:10.1016/j.lfs.2013.04.013.

[35] d'Alessio, P., Mirshahi, M., Bisson, J.-F., & Bene, M. (2014). Skin Repair Properties of d-Limonene and Perillyl Alcohol in Murine Models. Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry, 13(1), 29–35. doi:10.2174/18715230113126660021.

[36] Liu, H., Wang, C., Li, C., Qin, Y., Wang, Z., Yang, F., Li, Z., & Wang, J. (2018). A functional chitosan-based hydrogel as a wound dressing and drug delivery system in the treatment of wound healing. RSC Advances, 8(14), 7533–7549. doi:10.1039/c7ra13510f.

[37] Yap, K. M., Sekar, M., Seow, L. J., Gan, S. H., Bonam, S. R., Mat Rani, N. N. I., Lum, P. T., Subramaniyan, V., Wu, Y. S., Fuloria, N. K., & Fuloria, S. (2021). Mangifera indica (Mango): A Promising Medicinal Plant for Breast Cancer Therapy and Understanding Its Potential Mechanisms of Action. Breast Cancer: Targets and Therapy, Volume 13, 471–503. doi:10.2147/bctt.s316667.

[38] Alemdaroğlu, C., Değim, Z., Çelebi, N., Zor, F., Öztürk, S., & Erdoğan, D. (2006). An investigation on burn wound healing in rats with chitosan gel formulation containing epidermal growth factor. Burns, 32(3), 319–327. doi:10.1016/j.burns.2005.10.015.

[39] Buosi, F. S., Alaimo, A., Di Santo, M. C., Elías, F., García Liñares, G., Acebedo, S. L., Castañeda Cataña, M. A., Spagnuolo, C. C., Lizarraga, L., Martínez, K. D., & Pérez, O. E. (2020). Resveratrol encapsulation in high molecular weight chitosan-based nanogels for applications in ocular treatments: Impact on human ARPE-19 culture cells. International Journal of Biological Macromolecules, 165, 804–821. doi:10.1016/j.ijbiomac.2020.09.234.

[40] Stricker-Krongrad, A. H., Alikhassy, Z., Matsangos, N., Sebastian, R., Marti, G., Lay, F., & Harmon, J. W. (2018). Efficacy of chitosan-based dressing for control of bleeding in excisional wounds. Eplasty, 18, 122-130.

[41] Singh, R., Shitiz, K., & Singh, A. (2017). Chitin and chitosan: biopolymers for wound management. International Wound Journal, 14(6), 1276–1289. doi:10.1111/iwj.12797.

[42] Ferreira, P. G., Ferreira, V. F., da Silva, F. de C., Freitas, C. S., Pereira, P. R., & Paschoalin, V. M. F. (2022). Chitosans and Nanochitosans: Recent Advances in Skin Protection, Regeneration, and Repair. Pharmaceutics, 14(6), 1307. doi:10.3390/pharmaceutics14061307.

[43] Alharbi, A.M., Alharbi, T.M., Alqahtani, M.S., Elfasakhany, F.M., Afifi, I.K., Rajeh, M. T., ... & Kenawi, L.M.M. (2023). A Comparative Evaluation of Antibacterial Efficacy of Moringa oleifera Leaf Extract, Octenidine Dihydrochloride, and Sodium Hypochlorite as Intracanal Irrigants against Enterococcus faecalis: An In Vitro Study. International Journal of Dentistry, 7690497.

[44] Kou, X., Li, B., Olayanju, J. B., Drake, J. M., & Chen, N. (2018). Nutraceutical or pharmacological potential of Moringa oleifera Lam. Nutrients, 10(3), 343. doi:10.3390/nu10030343.

[45] Predoi, D., Ciobanu, C. S., Iconaru, S. L., Raaen, S., Badea, M. L., & Rokosz, K. (2022). Physicochemical and Biological Evaluation of Chitosan-Coated Magnesium-Doped Hydroxyapatite Composite Layers Obtained by Vacuum Deposition. Coatings, 12(5), 702. doi:10.3390/coatings12050702.

[46] Zhang, Y., Chan, H. F., & Leong, K. W. (2013). Advanced materials and processing for drug delivery: The past and the future. Advanced Drug Delivery Reviews, 65(1), 104–120. doi:10.1016/j.addr.2012.10.003.

[47] Herb, M., & Schramm, M. (2021). Functions of ROS in macrophages and antimicrobial immunity. Antioxidants, 10(2), 1–39. doi:10.3390/antiox10020313.

[48] Flora, S. J. S., & Pachauri, V. (2010). Chelation in metal intoxication. International Journal of Environmental Research and Public Health, 7(7), 2745–2788. doi:10.3390/ijerph7072745.

[49] Islam, M. M., Shahruzzaman, M., Biswas, S., Nurus Sakib, M., & Rashid, T. U. (2020). Chitosan based bioactive materials in tissue engineering applications-A review. Bioactive Materials, 5(1), 164–183. doi:10.1016/j.bioactmat.2020.01.012.

[50] Xu, J., Wise, J. T. F., Wang, L., Schumann, K., Zhang, Z., & Shi, X. (2017). Dual roles of oxidative stress in metal carcinogenesis. Journal of Environmental Pathology, Toxicology and Oncology, 36(4), 345–376. doi:10.1615/JEnvironPatholToxicolOncol.2017025229.

[51] Subramaniam, T., Fauzi, M. B., Lokanathan, Y., & Law, J. X. (2021). The role of calcium in wound healing. International Journal of Molecular Sciences, 22(12), 6486. doi:10.3390/ijms22126486.

[52] Navarro-Requena, C., Pérez-Amodio, S., Castano, O., & Engel, E. (2018). Wound healing-promoting effects stimulated by extracellular calcium and calcium-releasing nanoparticles on dermal fibroblasts. Nanotechnology, 29(39), 395102. doi:10.1088/1361-6528/aad01f.

[53] Lansdown, A. B. G. (2002). Calcium: A potential central regulator in wound healing in the skin. Wound Repair and Regeneration, 10(5), 271–285. doi:10.1046/j.1524-475X.2002.10502.x.

[54] Özsu, N., & Monteiro, A. (2017). Wound healing, calcium signaling, and other novel pathways are associated with the formation of butterfly eyespots. BMC Genomics, 18(1), 1–14. doi:10.1186/s12864-017-4175-7.

[55] Kawai, K., Larson, B. J., Ishise, H., Carre, A. L., Nishimoto, S., Longaker, M., & Lorenz, H. P. (2011). Calcium-based nanoparticles accelerate skin wound healing. PLoS One, 6(11), 27106. doi:10.1371/journal.pone.0027106.

[56] Kim, D. H., Lee, J. Y., Kim, Y. J., Kim, H. J., & Park, W. (2020). Rubi fructus water extract alleviates lps-stimulated macrophage activation via an ER stress-induced calcium/chop signaling pathway. Nutrients, 12(11), 3577. doi:10.3390/nu12113577.

[57] Klein, G. L. (2018). The role of calcium in inflammation-associated bone resorption. Biomolecules, 8(3), 69. doi:10.3390/biom8030069.

[58] Younis, I. (2020). Role of oxygen in wound healing. Journal of Wound Care, 29(Sup5b), S4–S10. doi:10.12968/jowc.2020.29.Sup5b.S4.

[59] Guo, S., & DiPietro, L. A. (2010). Critical review in oral biology & medicine: Factors affecting wound healing. Journal of Dental Research, 89(3), 219–229. doi:10.1177/0022034509359125.

[60] Liu, M., Wang, X., Li, H., Xia, C., Liu, Z., Liu, J., Yin, A., Lou, X., Wang, H., Mo, X., & Wu, J. (2021). Magnesium oxide-incorporated electrospun membranes inhibit bacterial infections and promote the healing process of infected wounds. Journal of Materials Chemistry B, 9(17), 3727–3744. doi:10.1039/d1tb00217a.

[61] Politis, C., Schoenaers, J., Jacobs, R., & Agbaje, J. O. (2016). Wound Healing Problems in the Mouth. Frontiers in Physiology, 7. doi:10.3389/fphys.2016.00507.

[62] Peacock, M. (2021). Phosphate Metabolism in Health and Disease. Calcified Tissue International, 108(1), 3–15. doi:10.1007/s00223-020-00686-3.

[63] Król, A., Mizerna, K., & Bożym, M. (2020). An assessment of pH-dependent release and mobility of heavy metals from metallurgical slag. Journal of Hazardous Materials, 384, 121502. doi:10.1016/j.jhazmat.2019.121502.

[64] Mghaiouini, R., Abdelhadi, M., Hairch, Y., Saifaoui, D., Salah, M., Abderrahmane, E., Chahid, E. G., Bensemlali, M., Belhora, F., El Mouden, M., Monkade, M., & El Bouari, A. (2022). The effect of low frequency of electromagnetic field on the freezing and cooling process of water. Materials Today: Proceedings, 66, 85–94. doi:10.1016/j.matpr.2022.03.455.

[65] Nunthanid, J., Puttipipatkhachorn, S., Yamamoto, K., & Peck, G. E. (2001). Physical properties and molecular behavior of chitosan films. Drug Development and Industrial Pharmacy, 27(2), 143–157. doi:10.1081/DDC-100000481.

[66] Sirkka, T., Skiba, J. B., & Apell, S. P. (2016). Wound pH depends on actual wound size. arXiv preprint arXiv:1601.06365. doi:10.1111/j.1365-2621.2006.tb15624.x.

[67] Boron, W. F. (2004). Regulation of intracellular pH. American Journal of Physiology - Advances in Physiology Education, 28(4), 160–179. doi:10.1152/advan.00045.2004.

[68] Sirkka, T., Skiba, J., & Apell, S., (2016). Wound pH depends on actual wound size. arXiv preprint arXiv:1601.06365. doi:10.48550/arXiv.1601.06365.

[69] Harguindey, S., Orive, G., Luis Pedraz, J., Paradiso, A., & Reshkin, S. J. (2005). The role of pH dynamics and the Na+/H+ antiporter in the etiopathogenesis and treatment of cancer. Two faces of the same coin—one single nature. Biochimica et Biophysica Acta (BBA) - Reviews on Cancer, 1756(1), 1–24. doi:10.1016/j.bbcan.2005.06.004.

[70] Li, Z., Zhao, Y., Liu, H., Ren, M., Wang, Z., Wang, X., Liu, H., Feng, Y., Lin, Q., Wang, C., & Wang, J. (2021). pH-responsive hydrogel loaded with insulin as a bioactive dressing for enhancing diabetic wound healing. Materials & Design, 210, 110104. doi:10.1016/j.matdes.2021.110104.

[71] Nagoba, B. S., Suryawanshi, N. M., Wadher, B., & Selkar, S. (2015). Acidic environment and wound healing: a review. Wounds-a Compendium of Clinical Research and Practice, 27(1), 5-11.

[72] Kant, V., Jangir, B. L., & Kumar, V. (2020). Gross and histopathological effects of dimethyl sulfoxide on wound healing in rats. Wound Medicine, 30, 100194. doi:10.1016/j.wndm.2020.100194.

[73] Hebling, J., Bianchi, L., Basso, F. G., Scheffel, D. L., Soares, D. G., Carrilho, M. R. O., Pashley, D. H., Tjäderhane, L., & De Souza Costa, C. A. (2015). Cytotoxicity of dimethyl sulfoxide (DMSO) in direct contact with odontoblast-like cells. Dental Materials, 31(4), 399–405. doi:10.1016/j.dental.2015.01.007.

[74] Koland, M., Vijayanarayana, K., Charyulu, Rn., & Prabhu, P. (2011). In vitro and in vivo evaluation of chitosan buccal films of ondansetron hydrochloride. International Journal of Pharmaceutical Investigation, 1(3), 164. doi:10.4103/2230-973x.85967.

[75] Zhao, X., Wu, H., Guo, B., Dong, R., Qiu, Y., & Ma, P. X. (2017). Antibacterial anti-oxidant electroactive injectable hydrogel as self-healing wound dressing with hemostasis and adhesiveness for cutaneous wound healing. Biomaterials, 122, 34–47. doi:10.1016/j.biomaterials.2017.01.011.

[76] Mikušová, V., & Mikuš, P. (2021). Advances in chitosan-based nanoparticles for drug delivery. International Journal of Molecular Sciences, 22(17). doi:10.3390/ijms22179652.

[77] Daza, L. D., Eim, V. S., & Váquiro, H. A. (2021). Influence of ulluco starch concentration on the physicochemical properties of starch–chitosan biocomposite films. Polymers, 13(23), 4232. doi:10.3390/polym13234232.

[78] Spinks, G. M., Lee, C. K., Wallace, G. G., Kim, S. I., & Kim, S. J. (2006). Swelling behavior of chitosan hydrogels in ionic liquid-water binary systems. Langmuir, 22(22), 9375–9379. doi:10.1021/la061586r.

[79] Ostrowska-Czubenko, J., Gierszewska, M., & Pieróg, M. (2015). pH-responsive hydrogel membranes based on modified chitosan: water transport and kinetics of swelling. Journal of Polymer Research, 22(8). doi:10.1007/s10965-015-0786-3.

[80] Kayserilioğlu, B. Ş., Bakir, U., Yilmaz, L., & Akkaş, N. (2003). Use of xylan, an agricultural by-product, in wheat gluten based biodegradable films: Mechanical, solubility and water vapor transfer rate properties. Bioresource Technology, 87(3), 239–246. doi:10.1016/S0960-8524(02)00258-4.

[81] Cao, Z., Luo, X., Zhang, H., Fu, Z., Shen, Z., Cai, N., Xue, Y., & Yu, F. (2016). A facile and green strategy for the preparation of porous chitosan-coated cellulose composite membranes for potential applications as wound dressing. Cellulose, 23(2), 1349–1361. doi:10.1007/s10570-016-0860-y.

[82] Zhang, Z. H., Han, Z., Zeng, X. A., Xiong, X. Y., & Liu, Y. J. (2015). Enhancing mechanical properties of chitosan films via modification with vanillin. International Journal of Biological Macromolecules, 81, 638–643. doi:10.1016/j.ijbiomac.2015.08.042.

[83] Pinto, E. P., Tavares, W. D. S., Matos, R. S., Ferreira, A. M., Menezes, R. P., Da Costa, M. E. H. M., De Souza, T. M., Ferreira, I. M., De Sousa, F. F. O., & Zamora, R. R. M. (2018). Influence of low and high glycerol concentrations on wettability and flexibility of chitosan biofilms. Quimica Nova, 41(10), 1109–1116. doi:10.21577/0100-4042.20170287.

[84] Rachtanapun, P., Klunklin, W., Jantrawut, P., Jantanasakulwong, K., Phimolsiripol, Y., Seesuriyachan, P., Leksawasdi, N., Chaiyaso, T., Ruksiriwanich, W., Phongthai, S., Sommano, S. R., Punyodom, W., Reungsang, A., & Ngo, T. M. P. (2021). Characterization of chitosan film incorporated with curcumin extract. Polymers, 13(6). doi:10.3390/polym13060963.

[85] Malm, M., Liceaga, A. M., San Martin-Gonzalez, F., Jones, O. G., Garcia-Bravo, J. M., & Kaplan, I. (2021). Development of Chitosan Films from Edible Crickets and Their Performance as a Bio-Based Food Packaging Material. Polysaccharides, 2(4), 744–758. doi:10.3390/polysaccharides2040045.

[86] Zhang, Z., & Angst, U. (2020). A Dual-Permeability Approach to Study Anomalous Moisture Transport Properties of Cement-Based Materials. Transport in Porous Media, 135(1), 59–78. doi:10.1007/s11242-020-01469-y.

[87] Dai, Z., Ansaloni, L., Ryan, J. J., Spontak, R. J., & Deng, L. (2018). Nafion/IL hybrid membranes with tuned nanostructure for enhanced CO2 separation: Effects of ionic liquid and water vapor. Green Chemistry, 20(6), 1391–1404. doi:10.1039/c7gc03727a.

[88] Palivan, C. G., Goers, R., Najer, A., Zhang, X., Car, A., & Meier, W. (2016). Bioinspired polymer vesicles and membranes for biological and medical applications. Chemical Society Reviews, 45(2), 377–411. doi:10.1039/c5cs00569h.

[89] Liu, Y., Cai, Z., Sheng, L., Ma, M., Xu, Q., & Jin, Y. (2019). Structure-property of crosslinked chitosan/silica composite films modified by genipin and glutaraldehyde under alkaline conditions. Carbohydrate Polymers, 215, 348–357. doi:10.1016/j.carbpol.2019.04.001.

[90] Jakfar, S., Lin, T. C., Chen, Z. Y., Yang, I. H., Gani, B. A., Ningsih, D. S., Kusuma, H., Chang, C. T., & Lin, F. H. (2022). A Polysaccharide Isolated from the Herb Bletilla striata Combined with Methylcellulose to Form a Hydrogel via Self-Assembly as a Wound Dressing. International Journal of Molecular Sciences, 23(19), 12019. doi:10.3390/ijms231912019.

[91] Wang, Y., Cheng, G., Wu, W., Qiao, Q., Li, Y., & Li, X. (2015). Effect of pH and chloride on the micro-mechanism of pitting corrosion for high strength pipeline steel in aerated NaCl solutions. Applied Surface Science, 349, 746–756. doi:10.1016/j.apsusc.2015.05.053.

[92] Tong, X., Sheng, G., Yang, D., Li, S., Lin, C. W., Zhang, W., Chen, Z., Wei, C., Yang, X., Shen, F., Shao, Y., Wei, H., Zhu, Y., Sun, J., Kaner, R. B., & Shao, Y. (2022). Crystalline tetra-aniline with chloride interactions towards a biocompatible supercapacitor. Materials Horizons, 9(1), 383–392. doi:10.1039/d1mh01081f.

[93] Reichardt, A. (2014). Tissue engineering of human heart valves: prerequisites for a reproducible fabrication process. PhD Thesis, Technische Universität Berlin, Berlin, Germany.

[94] Simi, C. K., & Abraham, T. E. (2010). Biodegradable biocompatible xyloglucan films for various applications. Colloid and Polymer Science, 288(3), 297–306. doi:10.1007/s00396-009-2151-8.

[95] Zhang, L., Zhang, Z., Chen, Y., Ma, X., & Xia, M. (2021). Chitosan and procyanidin composite films with high antioxidant activity and pH responsivity for cheese packaging. Food Chemistry, 338, 128013. doi:10.1016/j.foodchem.2020.128013.

[96] Pragati, S., Ashok, S., & Kuldeep, S. (2009). Recent advances in periodontal drug delivery systems. International Journal of Drug Delivery, 1(1), 1–14. doi:10.5138/ijdd.2009.0975.0215.01001.

[97] Venkataramani, S., Truntzer, J., & Coleman, D. R. (2013). Thermal stability of high concentration lysozyme across varying pH: A Fourier Transform Infrared study. Journal of Pharmacy and Bioallied Sciences, 5(2), 148–153. doi:10.4103/0975-7406.111821.

[98] Bhargav, H. S., Shastri, S. D., Poornav, S. P., Darshan, K. M., & Nayak, M. M. (2016). Measurement of the Zone of Inhibition of an Antibiotic. 2016 IEEE 6th International Conference on Advanced Computing (IACC). doi:10.1109/iacc.2016.82.

[99] Dai, T., Tanaka, M., Huang, Y. Y., & Hamblin, M. R. (2011). Chitosan preparations for wounds and burns: Antimicrobial and wound-healing effects. Expert Review of Anti-Infective Therapy, 9(7), 857–879. doi:10.1586/eri.11.59.

[100] Khattak, R. Z., Nawaz, A., Alnuwaiser, M. A., Latif, M. S., Rashid, S. A., Khan, A. A., & Alamoudi, S. A. (2022). Formulation, In Vitro Characterization and Antibacterial Activity of Chitosan-Decorated Cream Containing Bacitracin for Topical Delivery. Antibiotics, 11(9). doi:10.3390/antibiotics11091151.

[101] Yan, D., Li, Y., Liu, Y., Li, N., Zhang, X., & Yan, C. (2021). Antimicrobial properties of chitosan and chitosan derivatives in the treatment of enteric infections. Molecules, 26(23). doi:10.3390/molecules26237136.

[102] Alven, S., & Aderibigbe, B. A. (2020). Chitosan and cellulose-based hydrogels for wound management. International Journal of Molecular Sciences, 21(24), 1–30. doi:10.3390/ijms21249656.

[103] Yilmaz Atay, H. (2019). Antibacterial Activity of Chitosan-Based Systems. Functional Chitosan. Springer, Singapore. doi:10.1007/978-981-15-0263-7_15.