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# Preparation and characterization of methoxy-poly(ethylene glycol) side chain grafted onto chitosan as a wound dressing film

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**ABSTRACT:** Chitosan has received extensive attention as a biomedical material; however, the poor solubility of chitosan is the major limiting factor in its utilization. In this study, chitosan-based biomaterials with improved aqueous solubility were synthesized. Two molecular weights (750 Da and 2000 Da) of methoxypoly(ethylene glycol) (mPEG) were grafted onto chitosan (mPEG-g-chitosan) to form a ~100-$\mu$m-thick plastic film as a wound dressing. The chemical structures of the mPEG-g-chitosan copolymers were confirmed using Fourier transform infrared spectroscopy (FTIR), and the thermal properties were characterized using thermogravimetry analysis (TGA). Their microstructures were observed using scanning electron microscopy (SEM). The other properties were analyzed via the swelling ratio, tensile strength, elongation, and water vapor transmission rate (WVTR). Biocompatibility evaluations through biodegradability, cytotoxicity, and antimicrobial effect studies were also performed. The obtained mPEG-g-chitosan copolymers were soluble in slightly acidic aqueous solutions (pH~6.5) at a concentration of 10 wt %. The optimal mPEG-g-chitosan hydrogels had swelling ratios greater than 100% and WVTRs greater than 2000 g/m$^2$/day. Their performance against *Staphylococcus aureus* will be subjected to further improvements with respect to medical applications. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42340.

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## INTRODUCTION

Wound dressings are generally composed of two components, a film-forming polymer and an anti-microbial substance.[1] The requirements of a suitable biomaterial for wound dressings are the ability to form a flexible non-irritating thin polymer film that is innocuous to the wound, conformation to the skin surface, possession of a high moisture transmission rate to permit wound excretions to pass through the film and evaporate moisture from the covered surface, and prevention of bacterial and dirt invasion.[1–3] Chitosan wound dressings have many advantageous properties, such as biocompatibility, biodegradability, non-toxicity, accelerated wound healing properties, and antimicrobial activities.[3,4] Chitosan itself gradually degrades into *N*-acetyl-b-D-glucosamine, which causes fibroblast proliferation, helps to stimulate collagen and hyaluronic acid formation in the wound, accelerates wound healing, and prevents scarring.[5] Chitin consists of linear structures of 2-acetamido-2-deoxy-$\beta$-D-glucose through $\beta(1\rightarrow4)$ linkages. Chitosan is the chitin derivative produced by the N-deacetylation process, resulting in an amino group at the C-2 position on its backbone.[6–10] The distinction between chitin and chitosan is mainly based on the degree of deacetylation; however, these two substances are not differentiated by a consistent naming convention or norm. Generally, molecules with less than 40% deacetylation are called chitin, and those with greater than 60% deacetylation are called chitosan.[11,12] Chitosan is a semi-crystalline polymer, a weak base that is insoluble in water, alkali or aqueous solutions above pH 7, and common organic solvents due to its stable and rigid crystalline structure. A small amount of chitosan can be protonated upon dissolution in an aqueous acidic medium at pH $< 6.5$;[13] however, when the dissolved chitosan possesses a large positive charge on its $-NH_3^+$ groups, the resultant soluble polysaccharide is positively charged. Hence, improving the solubility of chitosan is crucial if this plentiful resource is to be utilized across a wide pH range. Various chemical modifications have been used to improve its solubility at neutral and alkaline pH. Chitosan has three reactive groups, i.e. a primary hydroxyl

group at C-6 and a secondary hydroxyl group at C-3 on each repeated unit, and an amino group at C-2 on each deacetylated unit. There have been two major approaches documented in literature toward improving the solubility of chitosan at neutral pH. First is to chemically derivatize chitosan, such as carboxymethyl chitosan, *N*-sulfofuryl chitosan, 5-methyl pyrrolidinone chitosan, dicarboxymethyl chitosan, and quaternized chitosan have been reported, with a solubility range of 0.3–1.0% obtained.[14] The second approach uses chitosan with an average 50% deacetylation prepared by homogenous processing of chitin.[15]

In the field of biology, polyethylene glycol (PEG) has low toxicity; exhibits nonspecific adsorption of proteins, cells, and bacteria;[16,17] is resistant to degrading enzymes and mammalian cells;[18] and possesses low antigenicity and low immunogenicity.[19] PEG is a fascinating synthetic polymer that also has excellent water solubility and biodegradability. Therefore, PEG has been used in many types of applications.[20] Suyatma *et al.*[21] reported that the mechanical properties of chitosan films could be improved by blending with PEG oligomers as a plasticizer. In addition, the PEG-cross-linked chitosan hydrogel films with different contents and molecular weight of PEG were prepared and then made into films, which were used to explore the swelling and biodegradability of this material.[22,23] Sugimoto *et al.*[24] used the reductive amination of PEG-aldehyde in an aqueous organic acid as a typical method for grafting PEG onto chitosan. They found that the solubility of chitosan-g-PEG in water was dependent on the molecular weight of the PEG, the weight ratio of PEG in the hybrid and the degree of substitution. Other experimental results show that the biodegradability increases with PEG content, and the weight loss increases with PEG molecular weight.[25] In our previous research,[26,27] PEG was used to reinforce chitosan-based wound dressings through the formation of ester and amide linkages. The reinforced PEG/chitosan (RPC) hydrogel exhibited significant enhancements in tensile modulus and elongation compared with neat chitosan; however, this type of RPC was not soluble in slightly acidic aqueous solutions.

Accordingly, the aim of the present study is to provide two types of different molecular weight mPEG molecules grafted onto chitosan as side chains (mPEG-g-chitosan). It is expected that the chitosan molecules will retain their original features and have increased water solubility. Here, we describe the synthesis and physicochemical characterization of chitosan-grafted mPEG copolymers. Another objective herein is to form an antimicrobial film that is non-toxic and flexible, possesses optimal mechanical properties, is air permeable and demonstrates *in vitro* degradation. In addition to *in vitro* cell viability, the effects of the mPEG-g-chitosan films on the epidermis were also evaluated using 3T3 fibroblasts.

## MATERIALS AND METHODS

### Reagents

Chitosan (average molecular weight: 300 kDa, degree of deacetylation: 97%, and polydispersity index: 1.47) was purchased from G-HT Co., Hsinchu, Taiwan. Methoxypoly(ethylene glycol) (mPEG, average molecular weight: 750 and 2000 Da), succinic anhydrate, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), and N-hydroxysulfosuccinimide (NHS) were purchased from Acros, Geel, Belgium. Acetic acid ($CH_3COOH$), sodium hydroxide (NaOH), ethanol ($C_2H_5OH$), phosphate buffered saline (PBS), and all other chemicals were purchased from Sigma-Aldrich Corp., St. Louis, MO, USA.

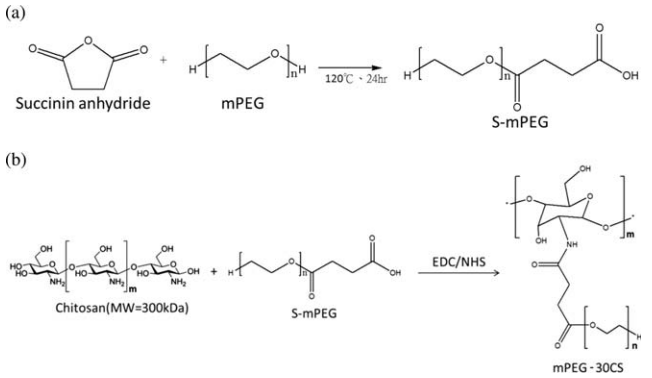


**Figure 1.** Schematic representation of (a) the modification of mPEG with succinic anhydrate (S-mPEG) and (b) the amide bonds formed in S-mPEG-grafted chitosan (30 kD) (mPEG-30CS).

### Preparation of Succinic-Anhydride-Modified mPEG (S-mPEG)

Chitosan-based films with higher solubility in slightly acidic aqueous solutions, composed of chitosan and mPEG, were synthesized through the formation of simple ester and amide linkages. First, mPEG was modified with succinic anhydrate through an esterification reaction, as shown in Figure 1(a). S-mPEG was prepared by the reaction of mPEG with succinic anhydride, which was performed at 120°C for 24 h, and the end product was a PEG-acid with carboxylic acid groups at one end of the PEG. Fourier transform infrared spectroscopy (FTIR) was used to monitor the response of succinic-anhydride-modified mPEG (S-mPEG) to ensure complete reaction. Two different molecular weight PEG molecules (MW=750, and 2000 Da) were grafted onto chitosan as side chains in this study.

### Preparation of mPEG-g-Chitosan

Films of mPEG-g-chitosan, which consisted of S-mPEG and various molar ratios of carboxylic acid groups from the PEG-acid to amine groups from chitosan ([$-NH_2$]/[–COOH] = 100/0, 95/5, 90/10, 85/15, and 80/20), were prepared as shown in Figure 1(b). The compositions of the mPEG-g-chitosan films are listed in Table I. A brief description of the procedure is as follows: to take a certain amounts of chitosan (300 kDa) was mixed with water to reach 4 wt %, acetic acid was slowly added dropwise with gentle stirring for 12 h until a uniform reaction was achieved (3 wt %). Next, the 10 g S-mPEG was completely dissolved in 30 mL of deionized water by sonication. Then, S-mPEG (750 or 2000 Da) solutions of 30.6 g were added to the 3 wt % chitosan solution of 100 g with acetic acid, and the mixture was stirred for 30 min to make it uniform. However, the mPEGs modified with chitosan were subject to amide bond formation using EDC/NHS as a coupling agent. The 1.3 times moles of NHS ([NHS]/[COOH] = 1.3) and EDC ([EDC]/[COOH] = 1.3) than COOH in an ethanol solution was added to the aforementioned solution of S-mPEGs/chitosan, and the

**Table I.** Description of the Neat Chitosan and S-mPEG Grafted Chitosan

| Nomenclature | Mw of mPEG (Da) | Molar ratio of reactive functional groups ($NH_2$ on Chitosan/COOH on grafted mPEG) | Wt % of Chitosan/ modified mPEG (%) |
|---|---|---|---|
| Chitosan | - | 100/0 | 100.00/0.00 |
| mPEG750-30C95S | 750 | 95/5 | 80.31/19.69 |
| mPEG750-30C90S | 750 | 90/10 | 65.89/34.11 |
| mPEG750-30C85S | 750 | 85/15 | 54.88/45.12 |
| mPEG750-30C80S | 750 | 80/20 | 46.20/53.80 |
| mPEG750-30C75S | 750 | 75/25 | 39.17/60.83 |
| mPEG2000-30C95S | 2000 | 95/5 | 60.47/39.53 |
| mPEG2000-30C90S | 2000 | 90/10 | 42.01/57.99 |
| mPEG2000-30C85S | 2000 | 85/15 | 31.33/68.67 |
| mPEG2000-30C80S | 2000 | 80/20 | 24.36/75.64 |
| mPEG2000-30C75S | 2000 | 75/25 | 19.45/80.55 |

reaction was left for 10 min at room temperature. The final mPEGs-30CS products were purified using a dialysis tube (3500 MWCO) against PBS for 1 day by five-exchange, followed by lyophilization. Solutions of 10 wt % mPEGs-30CS in acetic acid (pH~6.0) were obtained and poured onto a mold to form mPEGs-30CS films in a 60°C circulating oven. Then, a neat chitosan film was formed by solidification after immersing the chitosan solution (4 wt % in 1% acetic acid) into 1 N NaOH. The chitosan was neutralized with deionized water and then dried at 60°C.

### Characterization of the S-mPEG and mPEGs-30CS

**Chemical Structure.** FTIR (Spectrum 100, PerkinElmer, USA) was used to examine the peak variation of the amine and carboxylic acid groups of the S-mPEG and mPEGs-30CS. Powdered specimens were mixed with dry KBr at a ratio of 1/10 and then pressed into transparent discs.

**Microstructure Observation.** The morphology of each mPEGs-30CS type was observed by field emission-scanning electron microscopy (FE-SEM) using a JSM-6700F instrument (JOEL, Japan) at an accelerating voltage of 10 kV. All specimens were cryogenically fractured after immersion in liquid nitrogen for approximately 5 min. After dehydration for 1 h in a vacuum oven, the fractured surfaces were sputter coated with an ultrathin film of platinum to provide enhanced conductivity prior to SEM examination. The SEM images were recorded at magnifications of 2000×.

**Swelling Ratio.** To calculate the swelling ratios of the mPEGs-30CS and the neat chitosan, the dry films were weighed and then immersed in 0.05 M PBS at room temperature. After a specific time interval, the excess water was wiped on filter paper and the swollen specimens were reweighed. The swelling ratio was calculated using the following equation:

$$\text{Swelling ratio} = [(W_s - W_d)/W_d] \times 100\%, \quad (1)$$

where the swelling ratio is expressed as a percentage and $W_d$ and $W_s$ are the weights of the specimens before (dry) and after (swollen) being placed in PBS, respectively. The results are reported as the mean and standard deviation (mean ± SD) (n=6).

**Mechanical Properties.** A universal testing machine was used to determine the mechanical properties of the mPEGs-30CS and the neat chitosan, both in the dry state, by elongating the membrane specimens (6 cm in length and 1 cm in width) at a constant rate of 10 mm/min. The results are reported as the mean and standard deviation (n=6).

**Thermal Properties.** The thermal stability of the mPEGs-30CS samples and the neat chitosan were assessed using a thermal gravimetric analyzer (TGA; thermogravimetric analysis, PerkinElmer, TGA7), and the test was performed under nitrogen flow. All samples were preheated to 100°C for 15 min to ensure complete dehydration. The heating rate was 10°C/min from 100 to 800°C.

**Water Vapor Transmission Rate.** The water vapor transmission rates (WVTRs) across the mPEGs-30CS samples and neat chitosan were determined according to ASTM (F-1249-06). A permeability cup was used to determine the WVTR (Sheen Instruments, Surrey, England). The mPEGs-30CS were cut into 40×40 $mm^2$ squares with a thickness of 3 mm, mounted on the mouth of cylindrical aluminum cups (38 mm diameter) containing 10 mL water and placed in an incubator at 37°C. The WVTR was calculated using the following equation:

$$\text{WVTR} = (W_o - W_f)/(10^6 \times A) \text{g/m}^2\text{/day}, \quad (2)$$

where WVTR is expressed in $g/m^2/day$, A is the bottle mouth area in $mm^2$, and $W_o$ and $W_f$ are the weights in g of the device before and after being placed in an incubator for 24 h, respectively. The results are reported as the mean and standard deviation (n=3).

***In Vitro* Degradation.** The *in vitro* degradation experiments with the mPEGs-30CS and neat chitosan were performed according to ASTM (F-1635-95) by measuring the weight change over the degradation time under simulated physiological conditions. The dry specimens were weighed and then

immersed in 0.05 M PBS containing 58 or 100 units/mL of lysozyme at 37°C. After a specific time interval, the specimens were removed from the simulated physiological fluid and rinsed thoroughly with distilled water before being placed in a −20°C freezer. The specimens were freeze-dried for 24 h to remove excess water and then were reweighed. The remaining weight was calculated using the following equation:

$$\text{Weight Remaining } (\%) = 100 - [(W_o - W_d)/W_o \times 100], \quad (3)$$

where the remaining weight is expressed as a percentage, and $W_o$ and $W_d$ are the weights of the specimens before and after degradation for a specific time interval, respectively. The results are reported as the mean and standard deviation (n=6).

**Cytotoxicity.** The cytotoxicity of the mPEGs-30CS and neat chitosan was evaluated via the proliferation of 3T3 fibroblasts (Bioresource Collection and Research Center, Hsinchu, Taiwan). Briefly, pieces (1 cm × 1 cm) of each specimen, which were sterilized by an autoclave in distilled water for 15 min at 121°C, were placed in a 96-well polystyrene plate and treated with Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS), 1% (v/v) penicillin, and 1% (v/v) streptomycin. 3T3 fibroblasts ($1 \times 10^6$ cells/mL) were seeded onto each sample, which were subsequently maintained in a humidified atmosphere (5% $CO_2$, 100% humidity, 37°C). A tissue culture polystyrene (TCPS) dish was used as a control. The alamar blue assay was modified for cell proliferation estimation.[28] First, at indicated time points, the alamar blue solution (Invitrogen) was directly added into the culture wells, and the plate was further incubated at 37°C for 4 h. The absorbance of the wells was read at 570 and 600 nm using an ELISA plate reader (Molecular Devices, USA SpectraMax M2e) at the indicated time points (1 day, 3 days, and 5 days).

**Antibacterial Activity Evaluation.** The effect of an antimicrobial agent against bacterial grown in culture was tested according to the ISO 22196: 2007 (JIS Z-2801) standard. The material was tested for its antibacterial activity against *Staphylococcus aureus* (*S. aureus*) CCM 4516 as a representative Gram-positive bacterium. The size of test specimens was chosen as 10 mm×10 mm×0.5 mm. The specimens were placed in Petri dishes and inoculated with 0.4 mL of a bacterial culture containing $10^5$ to $10^6$ CFU/mL. The inoculums were covered with a polyester film (X-131 transparent copier film; Folex Imaging), and the Petri dishes were incubated at 37°C for 24 h in a humid chamber to prevent desiccation. After the incubation period, 20 mL of extraction solution (0.1% [v/v] Tween 20, 145 mM sodium chloride, 20.5 mM sodium phosphate; pH 7.4) was added to the Petri dishes, and the dishes were shaken for 2 min. Subsequently, serial dilutions of the extraction solution were spread on agar plates in triplicate and incubated at 37°C overnight. Colonies were counted visually, and the numbers of colony forming units (CFUs) per sample were determined.

The antibacterial activity R was calculated using eq. (4)

$$R = (U_t - U_0) - (A_t - U_0) = U_t - A_t \quad (4)$$

where R is the antibacterial activity, $U_0$ is the average of the logarithm of the number of viable bacteria (cells/cm$^2$) recovered from the untreated test specimens immediately after inoculation, $U_t$ is the average of the logarithm of the number of viable bacteria (cells/cm$^2$) recovered from the untreated test specimens immediately after 24 h, and $A_t$ is the average of the logarithm of the number of viable bacteria (cells/cm$^2$) recovered from the treated test specimens immediately after 24 h.

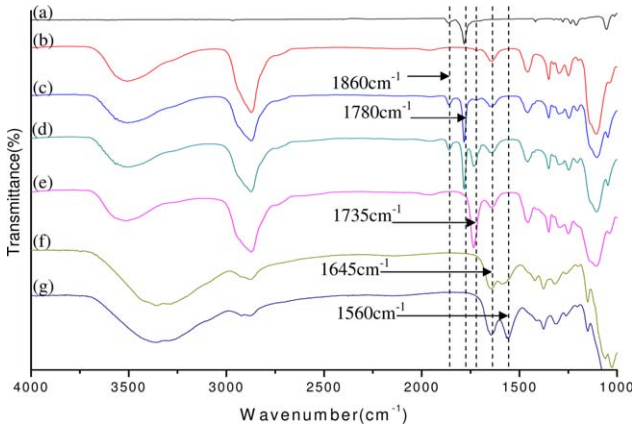


**Figure 2.** FTIR spectra of (a) succinic anhydride, (b) mPEG750, (c) succinic anhydride blended with mPEG750 and reacted for 0 min, (d) succinic anhydride blended with mPEG750 (S-mPEG750) and react for 4 h, (e) succinic anhydride blended with mPEG750 and react for 15 h (S-mPEG750), (f) chitosan, and (g) S-mPEG750 grafted with chitosan (30 kD) (75%) (mPEG750-30C75S). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

## RESULTS AND DISCUSSION

### Chemical Structure

mPEG was first modified with succinic anhydrate, which resulted in carboxylic acid groups at one end of the PEG forming S-mPEG, as shown in Figure 1(a). The chemical structure was further confirmed by FTIR spectroscopy, and the spectra of the starting materials and related products are shown in Figure 2(a-e). The characteristic peaks of succinic anhydride were observed at 1860 and 1780 cm$^{-1}$, which corresponded to the cyclic anhydride group absorption because cyclic anhydrides exhibit an intensive absorption band near 1780 cm$^{-1}$ and a weak band near 1860 cm$^{-1}$ due to the symmetric and asymmetric stretching of C=O, respectively.[29] The characteristic peaks of mPEG were observed at 3600∼3400 cm$^{-1}$ for the O-H absorption, 3000∼2840 cm$^{-1}$ for the C-H absorption and 1100 cm$^{-1}$ for the C-O stretching absorption.[30] The FTIR spectra of the S-mPEG featured similar characteristics to those of the parent compounds during 15 h; however, the two-peaks at 1860 and 1780 cm$^{-1}$ disappeared, and a new peak appeared at 1735 cm$^{-1}$ after esterification. This result suggested that the absorption of the product enhanced the carbonyl absorption peak at 1735 cm$^{-1}$ (C=O ester) and shifted it to a lower wavenumber value.

The mPEG-30CS copolymers were synthesized through the formation of an amide bond between S-mPEG and chitosan using EDC/NHS as a coupling agent. The characteristic peaks of neat chitosan [Figure 2(f)]were observed at 3300, 1150, and 1050 cm$^{-1}$, which corresponded to free amines (-$NH_2$), the glycosidic linkage between chitosan monomers (glucosamine), and

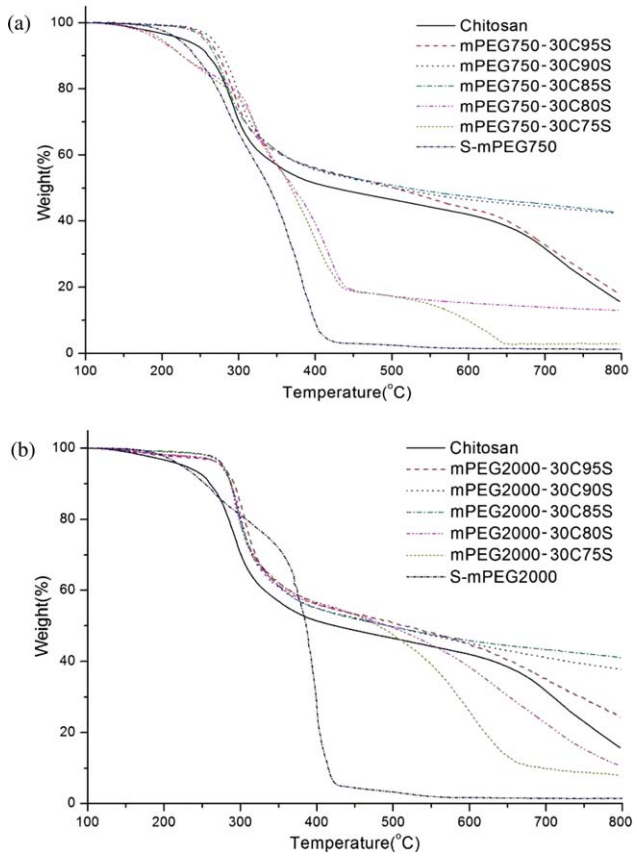


**Figure 3.** TGA heating curves of (a) the mPEG750-30CS series and (b) the mPEG2000-30CS series. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the ether bond (-C-O-C-) within the ring of the chitosan monomer, respectively.[31] The OH groups of chitosan have intermolecular and intramolecular hydrogen bonds, and the resulting absorption range (3600∼3400 $cm^{-1}$) is very broad. The FTIR spectra of the mPEG-30CS (mPEG750-30C75S) featured similar characteristics to the neat chitosan. However, the intensity of the N-H absorption peak at approximately 3300 $cm^{-1}$ for the mPEG750-30C75S should decrease relative to neat chitosan. This absorption peak was not clearly observed in each of the mPEG-30CS. However, two peaks were observed at 1645 and 1587 $cm^{-1}$ and were assigned to the amide linkage. The absorption intensity was altered by the incomplete deacetylation of chitosan and amide bond formation between chitosan and S-mPEG. Therefore, the intensity of the absorption peaks at 1645 and 1587 $cm^{-1}$ increased.

**TGA Analysis.** The mPEGs-30CS dressings were formed using polymers with high aqueous solubility to create a gel-based dressing. The solubility of the mPEGs-30CS system in acetic acid solutions (pH∼6.5) increased to 10 wt % compared with 4 wt % for chitosan. Therefore, the mPEGs-30CS system also helped to form films of stable thickness (∼100 $\mu$m). The TGA thermograms of neat chitosan and mPEGs-30CS are shown in Figure 3. All of these materials exhibited weight loss starting at approximately 85°C, which was caused by water loss. Chitosan showed a typical multi-step degradation pattern. The second rapid weight-loss of chitosan from 260 to 350°C was caused by the dehydration of the sugar unit, bond cleavage of the main chain, and decomposition of the acetyl and deacetylated units.[32] The final stage of weight loss of chitosan occurred above 600°C, mainly due to serious pyrolysis.

However, some of films containing mPEG (mPEGs-30CS) showed better thermal stability than the chitosan. The molar ratio of reactive functional groups ranged from 95/5∼85/15 for $NH_2$ on chitosan/COOH on grafted mPEG. Moreover, mPEG2000s-30CS showed the most stable matrix compared with the smaller molecular weight of mPEG750-30CS due to the better thermal stability of PEG2000 molecules than PEG750, which was also reflected in the enhanced thermal stability of mPEGs-30C85S. For example, the weight loss process of mPEG2000-30C85S also had main two phases, and it is clearly distinct from the weight loss curve of chitosan. The second stage of weight loss was observed between 300 and 360°C, and the final weight loss was noted as 46% of the residual amount at approximately 800°C.

**Table II.** Mechanical Properties of Neat Chitosan and Various S-mPEG Grafted Chitosan Films

| Nomenclature | Tensile modulus (MPa)[a] | Elongation (%)[a] |
|---|---|---|
| Chitosan | 102.16±11.19 | 27.03±1.04 |
| mPEG750-30C95S | 51.11±9.40 | 21.08±7.03 |
| mPEG750-30C90S | 37.43±2.69 | 17.25±5.55 |
| mPEG750-30C85S | 22.61±4.33 | 9.32±0.19 |
| mPEG750-30C80S | 16.89±3.55 | 9.14±1.75 |
| mPEG750-30C75S | 9.55±0.35 | 7.61±1.90 |
| mPEG2000-30C95S | 41.09±9.33 | 17.71±5.85 |
| mPEG2000-30C90S | 27.17±2.52 | 3.08±0.64 |
| mPEG2000-30C85S | 17.86±4.60 | 2.47±1.98 |
| mPEG2000-30C80S | 5.77±1.59 | 1.67±0.12 |
| mPEG2000-30C75S | 9.13±0.54 | 1.75±0.38 |

[a] Data are presented as mean ± SD (n = 6).

**Mechanical Properties**

The variation in mechanical properties with the molar ratio of reactive functional groups ($NH_2$ on chitosan/COOH on grafted mPEG) and the two PEG molecular weights is presented in Table II. The results show that the mechanical properties of chitosan with grafted mPEG rapidly decreased, and both the tensile strength and elongation decreased with increased mPEG weight percentage. Comparing the mPEG750-30CS series and the mPEG2000-30CS series, it was found that the larger molecular weight of mPEG (2000 Da) had a greater impact on the mechanical properties. In contrast, the decreasing rate of the mechanical properties for the mPEG2000-30CS series was higher than that of the mPEG750-30CS series. For example, the average tensile strength and elongation from mPEG750-30C85S and mPEG2000-30C85S were 22.61 MPa and 9.32%, and 17.86 MPa and 2.47%, respectively, likely because the functional

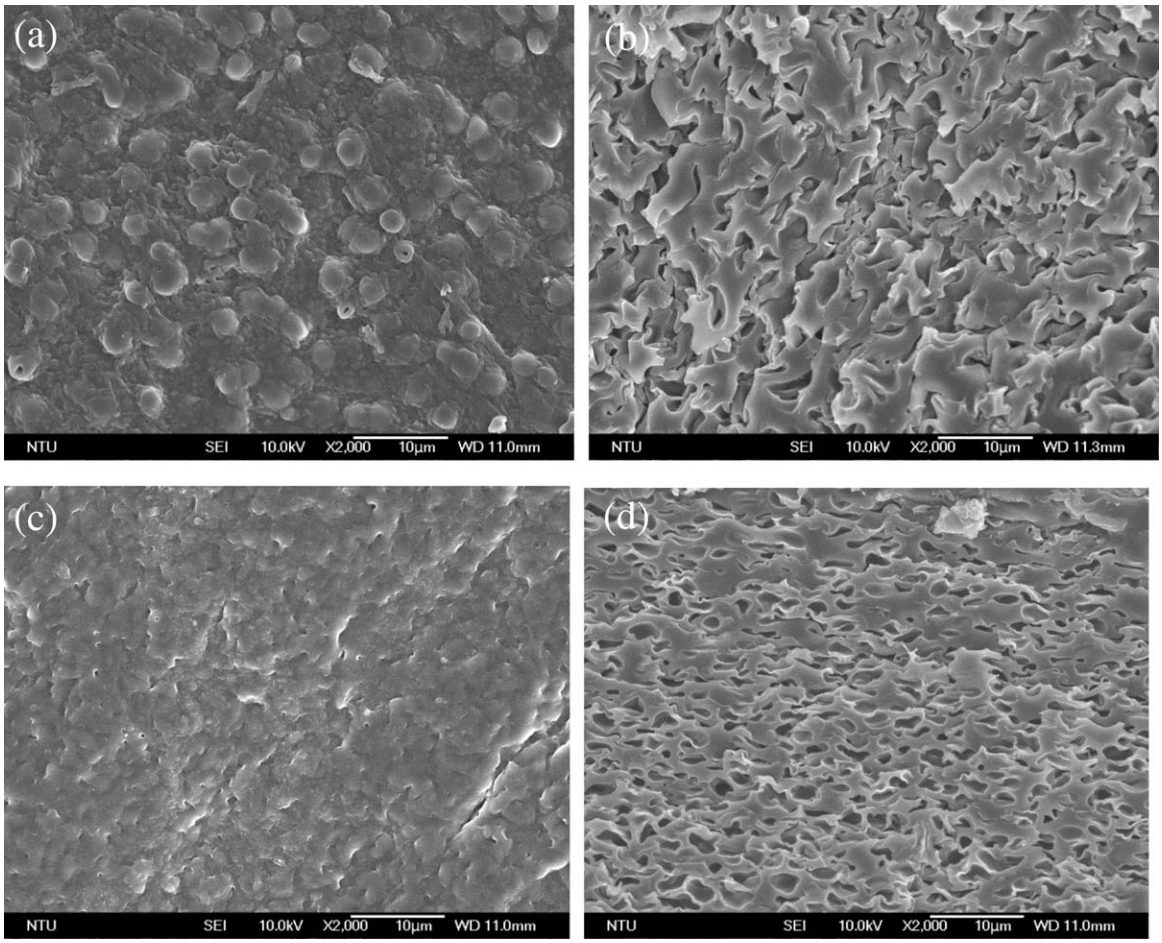


**Figure 4.** SEM images (2000× magnification) on the surface of chitosan (30 kD) (85%) reacted with mPEG (30C85S-mPEGs). The images show (a) the surface of mPEG750-30C85S, (b) the cross-section of mPEG750-30C85S, (c) the surface of mPEG2000-30C85S, and (d) the cross-section of mPEG2000-30C85S.

groups in the same proportion and the higher molecular weight of mPEG resulted in a higher weight percentage. Therefore, mPEG enhanced the steric hindrance in mPEG-30CS, caused intermolecular or intramolecular hydrogen bond destruction within the chitosan chains, and led to decreased tensile strength and elongation. Furthermore, the excessive methyl ether content of mPEG may have caused brittleness.

### Microstructure Observation

The physical behavior of the mPEGs-30CS films should be related to their internal structure. The surface morphologies of the mPEGs-30CS films revealed randomly dispersed clusters of domains in the polymer matrices, which are shown in the SEM images of Figure 3(a,c). However, the larger molecular weight of mPEG (2000 Da) influenced the domain sizes and connectivities. For mPEG750-30C85S, an isolated cluster region formed with a diameter of 2–4 $\mu$m. For mPEG2000-30C85S, the phase contrast of the hydrophilic domains increased and became less distinct from the film matrix; however, the domains remained segregated, with diameters of approximately 2 $\mu$m. The cross-sectional images of the mPEG2000-30C85S samples in Figure 4(b,d) also showed relatively uniform and small pore structure. In contrast, the smaller molecular weight of mPEG on mPEG750-30C85S induced the rougher and larger pores.

### Swelling Ratio

The hydrophilicities of the various mPEGs-30CS hydrogels and the chitosan were assessed by studying their water absorption capacity. A higher water absorption capacity contributes to improved wound healing because the wound dressing can absorb excess tissue fluid. The swelling ratios of the various films are presented in Figure 5. All films became saturated after immersion in phosphate buffer solution for 1 h. The swelling increased in proportion to the extent of mPEG grafting in the mPEGs-30CS polymers. However, the water absorption capacity of the mPEG2000-30CS films was better than the mPEG750-30CS films. mPEG has high hydrophilicity because the ether oxygen can combine with water molecules, causing the molecule to expand and dissolve.[33] The swelling ratio increased as the amount of PEG addition increased: mPEG750-30C95S (the lowest amount of PEG) had the lowest swelling ratio (approximately 168%), whereas mPEG2000-30C75S (the highest amount of PEG) had the greatest swelling ratio (approximately 470%), as illustrated in Figure 5(a,b). However, dramatic increases were

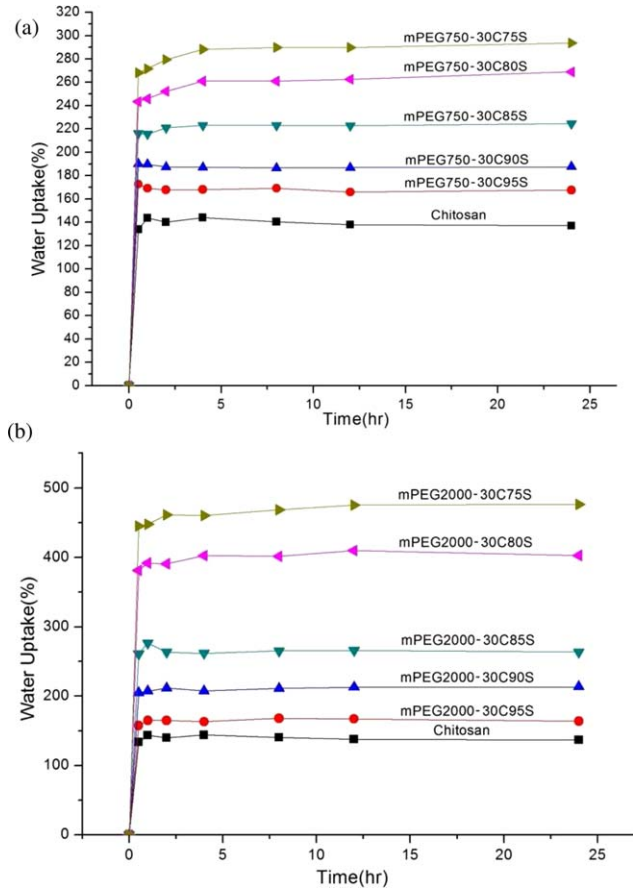


**Figure 5.** Swelling ratios of (a) the mPEG750-30CS series and (b) the mPEG2000-30CS series. The data are presented as the mean ± SD (n = 6). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

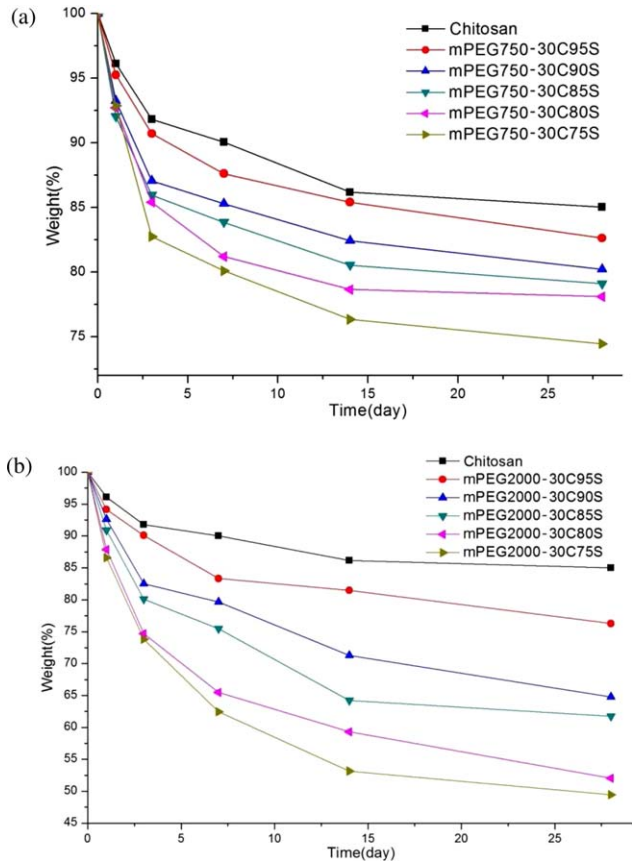


**Figure 6.** *In vitro* degradation was evaluated by the weight remaining. mPEG750-30CS series (a) and mPEG2000-30CS series (b). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

observed for mPEG2000-30C85S and mPEG750-30C85S, for which the swelling ratios were 255% and 220%, respectively.

### Water Vapor Transmission Rate

Table III presents the WVTRs of the various mPEGs-30CS hydrogels and the neat chitosan. The WVTR for the chitosan was approximately 1703±23 g/m$^2$/day; however, the values for the mPEG750-30CS and mPEG2000-30CS were between 1937±40~2258±56 g/m$^2$/day and 2315±47~2518±68 g/m$^2$/day, respectively. As either the amount or the molecular weight of PEG increased, the WVTR increased. These results were attributed to the hydrophilic properties of PEG.

An ideal wound dressing should maintain the evaporative water loss from the skin at an optimal rate. The evaporative water loss rate for normal skin is 204±12 g/m$^2$/day, whereas that for injured skin can range from 279±26 g/m$^2$/day for a first-degree burn to 5138±202 g/m$^2$/day for a granulating wound.[34] The water vapor permeability of a wound dressing should prevent excess dehydration, as well as the buildup of exudates. It is recommended that a rate between 2000 and 2500 g/m$^2$/day, which represent mid-range evaporative water loss rates from injured skin, would provide an adequate level of moisture without the risk of wound dehydration.[34,35] In this study, the WVTRs of the mPEG2000-30C85S and 30C85S-mPEG750 were approximately 2469±63 g/m$^2$/day and 2098±36 g/m$^2$/day, which falls in the range of the recommended evaporative water loss rate from

**Table III.** Water Vapor Transmission Rate (WVTR) and Antibacterial Properties of Neat Chitosan and Various S-mPEG Grafted Chitosan Films

| Nomenclature | WVTR (g/m$^2$/day)[a] | Antibacterial activity[b] |
|---|---|---|
| Chitosan | 1703±23 | 4.13 |
| mPEG750-30C95S | 1937±40 | 4.13 |
| mPEG750-30C90S | 2022±59 | 4.08 |
| mPEG750-30C85S | 2098±36 | 3.55 |
| mPEG750-30C80S | 2183±67 | 3.14 |
| mPEG750-30C75S | 2258±56 | 3.03 |
| mPEG2000-30C95S | 2315±47 | 3.82 |
| mPEG2000-30C90S | 2377±31 | 3.09 |
| mPEG2000-30C85S | 2469±63 | 2.88 |
| mPEG2000-30C80S | 2491±50 | 2.63 |
| mPEG2000-30C75S | 2518±68 | 2.41 |

[a] Data are presented as mean ± SD (n = 5).
[b] The values calculated with reference to population of bacteria recovered from the untreated specimens after inoculation (prior to incubation).

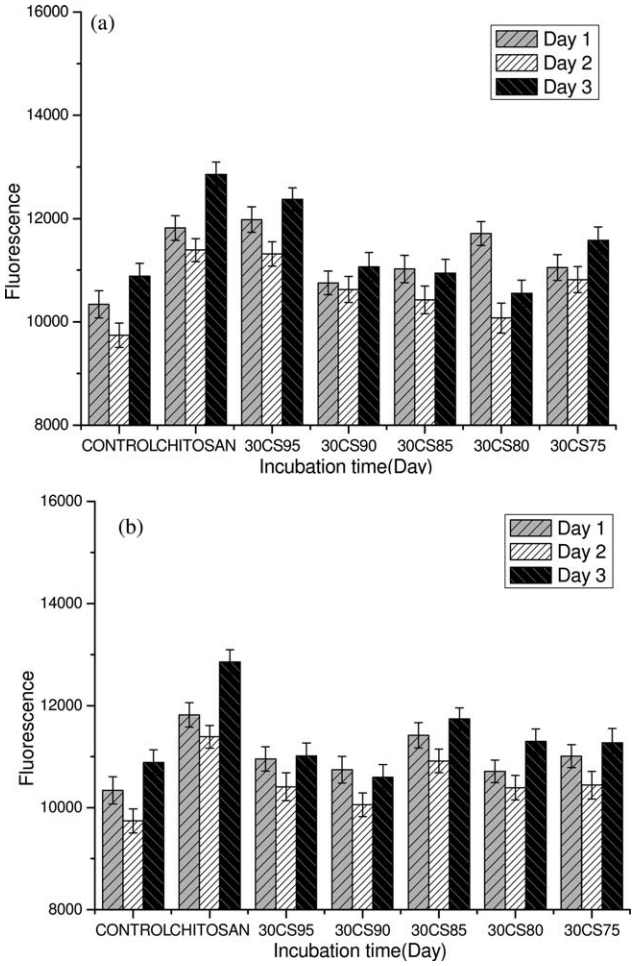


**Figure 7.** AlamarBlue® assay of 3T3 fibroblasts cultured on the (a) mPEG750-30CS series and (b) the mPEG2000-30CS series during 3 days. The data are presented as the mean ± S.D. (n = 3)

injured skin. Therefore, both mPEG2000-30C85S and mPEG750-30C85S could control the evaporative water loss at a suitable rate to yield a moist environment. All the mechanical properties, swelling ratio, and WVTR results indicate that mPEG2000-30C85S and mPEG750-30C85S are wound dressing candidates.

### *In Vitro* Degradation

The *in vitro* degradation results of the various mPEGs-30CS hydrogels and neat chitosan, given as the weight remaining after degradation, are shown in Figure 6. Overall, all the mPEGs-30CS hydrogels showed greater degradation than the neat chitosan after 28 days of incubation. As the amount or molecular weight of PEG increased, the degradation increased. The degradation results indicated that increasing the amount of PEG increased the swelling ratio and *in vitro* degradation rate. This phenomenon was attributed to the hydrophilicity of PEG. Because PEG is more hydrophilic than chitosan, which was determined via the swelling ratios (Table III), water and lysozymes were more highly attracted to the material. The increase in the presence of lysozymes resulted in the cleavage of the polymer chains and degradation.

### Cytotoxicity and Antibacterial Aactivity Evaluation

In this study, 3T3 fibroblasts were used to explore the effects of mPEGs-30CS copolymers on the cell viability of the epidermis. As shown in Figure 7, cytotoxic effects caused by the mPEGs-30CS copolymers on the 3T3 fibroblasts were illustrated by the alamar blue agent. The alamar blue reagent is an oxidized form of a redox indicator that is blue in color and non-fluorescent. When incubated with viable cells, the reagent changes color from blue to red and becomes fluorescent (600 nm). All of the mPEGs-30CS copolymers and neat chitosan had higher cell activity for 3T3 fibroblasts than the control during the culture period. It is well known that the positive charges of chitosan, which result from the protonation of the amino groups, facilitate the immobilization and growth of cells because most cellular membranes are negatively charged.[36] However, some research indicates that PEG-modified surfaces restrict the attachment of cells.[37] As indicated by the results, chitosan, the mPEG750-30CS series and the mPEG2000-30CS series showed higher or approximately the same cell activity compared with the control group. All materials improve cell growth and demonstrate good biocompatibility. However, all groups showed some declines at the second day, but the cells continued to grow at the third day.

The microorganisms and growth conditions used for antimicrobial testing are shown in Table III. The bacterial Log reduction value should be higher than 2.0 for mPEG-30CS films that can be categorized as having an effective antibacterial surface because this value is the minimal antibacterial activity according to ISO 22196:2007 and JIS Z2801:2000.[38] All the tested materials comply with this requirement for *S. aureus* CCM 4516. The performances of chitosan, mPEG750-30C95S, and mPEG750-30C90S illustrated that they are reasonable antibacterial dressings with respect to *S. aureus* (antibacterial activity > 4.08). Zheng *et al.* reported that the antimicrobial effect of chitosan on *S. aureus* was strengthened as the MW increased below 300 kDa.[39] However, the antibacterial activities of the mPEG750-30C85S and mPEG2000-30C85S films against *S. aureus* were 3.55 and 2.88, respectively. Their performances against *S. aureus* will be subjected to further improvements with respect to medical applications.

## CONCLUSION

Hydrogels with high aqueous solubility composed of chitosan and PEG were synthesized through the formation of simple ester and amide linkages. The molar ratio of reactive functional groups ranged between 95/5∼85/15 for $NH_2$ on chitosan/COOH to grafted mPEG. The mPEGs-30CS copolymers increased the solubility in acetic acid solutions (pH∼6.5) to 10 wt % compared with 4 wt % for chitosan. Therefore, mPEGs-30CS hydrogels also help to form a plastic film with a stable thickness of 100 $\mu$m for use as a wound dressing. Moreover, mPEG2000s-30CS showed the best thermal stability compared with the lower molecular weight mPEG750-30CS. However, the elongation and elastic modulus of the mPEGs-30CS hydrogel decreased as the amount of amide linkages from mPEG increased in the dry state. The average tensile strength and elongation from the candidate mPEG750-30C85S and mPEG2000-

30C85S films were 22.61 MPa and 9.32% and 17.86 MPa and 2.47%, respectively.

The other results indicated that increased amounts of PEG caused the swelling ratio and the *in vitro* degradation to increase. Furthermore, the WVTRs of the mPEG2000-30C85S and 30C85S-mPEG750 were approximately 2469±63 g/m$^2$/day and 2098±36 g/m$^2$/day, respectively, which falls in the range of the recommended evaporative water loss rate to reduce wound dehydration. Candidate mPEG750-30C85S and mPEG2000-30C85S film materials with activity against *S. aureus* will be subjected to further improvements with respect to medical applications. This study also provided a preliminary analysis of the interactions of chitosan-derived materials with epidermal cells, which indicated that chitosan-based materials may be used for regeneration of the epidermis.

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