ORIGINAL PAPER

Development of novel chitosan-poly(*N,N*-diethylacrylamide) IPN films for potential wound dressing and biomedical applications

Jude I. Ngadaonye · Luke M. Geever · John Killion · Clement L. Higginbotham

Abstract Novel interactive and thermoresponsive interpenetrating polymer network (IPN) films, which are transparent, permeable to oxygen, and have the potential to be easily stripped from a wound bed, were synthesised using rapid photopolymerisation and crosslinking of DEAAm in the presence of chitosan. This study provides the first evaluation and optimisation of a UV-polymerised chitosan–PDEAAm IPN composite film for application in wound dressings. FTIR spectroscopy and DSC analysis were used to initially characterise the resulting films. Modulated differential scanning calorimetry results showed that the dressings exhibited lower critical solution temperatures in the desired range, while the samples were also observed to undergo temperature-dependent swelling behaviour. This thermosensitive property would potentially allow the dressings to be easily detachable, which would enable frequent dressing changes if desired without causing further injury to healing tissues. Furthermore, the water content values recorded are in the typical and desired ranges for commercial wound dressings.

J. I. Ngadaonye · L. M. Geever · J. Killion ·
C. L. Higginbotham (✉)
Materials Research Group, Research Hub, Athlone Institute of Technology, Dublin Road,
Athlone, Co. Westmeath, Ireland
e-mail: chigginbotham@ait.ie

J. I. Ngadaonye
e-mail: jngadaonye@research.ait.ie

L. M. Geever
e-mail: lgeever@ait.ie

J. Killion
e-mail: jkillion@research.ait.ie

Introduction

Perhaps one of the most significant developments to change the nature of wound-dressing materials has been the concept of moist wound healing. Moist wound healing refers to the provision and maintenance of optimal hydration of the exposed tissues in the wound, as opposed to allowing or encouraging those tissues to dehydrate and dry out. Traditional dressings like gauze, bandages and cotton wool are dry and do not provide a moist wound environment. They also tend to become very adherent to the wound as fluid production diminishes, and are painful to remove. Gauze dressings also allow for excessive moisture evaporation, resulting in a dehydrated wound bed, and the gauze fibres are frequently entrapped in the wound. The use of dressings that keep wound tissues moist has been associated with increased healing rates, improved cosmesis, reduced pain, reduced infection, and reduced overall health care costs [1, 2].

Dressing removal, which has the potential to cause trauma to delicate healing tissue in wounds and the surrounding skin, is considered to be one of the most painful wound-care interventions [3]. Damage to the vulnerable underlying skin in wounds could also increase the risk of secondary infection. Hence, a dressing which can be readily stripped off the wound tissue is of the utmost importance in ensuring minimal patient discomfort. Generally, an effective wound dressing should not adhere excessively to the wound, in order to allow easy removal after healing. If the removal

of the dressing is not correctly performed, additional damage to the wound prolongs the recovery period. A high water content material can prevent adhesion problems, which in turn promotes the tissue regeneration process. In recent years, thermosensitive wound dressings have attracted attention with a view to achieving better control of the adhesion of dressings to wound beds [4–8].

Chitosan-based materials, produced in varying formulations, have been used in a number of wound-healing applications [9]. Detailed reviews of the applications of carboxymethyl derivatives of chitin and chitosan as drug delivery systems, in wound healing and repair, as antimicrobial agents, and in tissue engineering can be found in the literature [10–12]. Chitosan is a polysaccharide produced by the deacetylation of chitin (> 60 %), which is found in the exoskeletons of crustaceans. Chitin is the second most abundant natural biopolymer after cellulose, and is thus readily available. Chitosan itself can induce faster wound healing and produce smoother scarring [13].

Poly(N,N-diethylacrylamide) (PDEAAm) undergoes reversible hydration–dehydration changes in response to changes in temperature around their LCST (28–30 °C) in aqueous media. Its capacity to store and release active agents has made PDEAAm an attractive candidate for controlled drug delivery [14]. Hydrogels in the hydrated state are usually too soft, posing significant handling difficulties. There are various ways to improve the mechanical properties of hydrogels, including copolymerisation with hydrophobic monomers [15, 16] and the formation of interpenetrating polymer networks (IPNs) using flexible polymers [17, 19, 20]. The formation of IPNs, instead of synthesising new types of polymers, constitutes the most useful method of improving the properties of the individual components in order to satisfy the requirements of specific applications [18]. IPNs are a network of two polymers, at least one of which is crosslinked or synthesised or synthesised and crosslinked in the presence of the other. By incorporating PDEAAm into the film network, it is hypothesised that the dressing would adhere well to healing tissue when the polymer becomes slightly hydrophobic at body temperature, provided the LCST of the dressing is below this temperature. When a change or removal of the dressing is required, applying a low-temperature treatment (lower than the LCST of PDEAAm) to the dressing will make it more hydrophilic, so that it absorbs more water, significantly reducing the adhesiveness and reducing the risk of potential damage to fragile skin.

Poly(N-isopropylacrylamide) (PNIPAAm) has received huge attention in the literature as the polymer of choice for assigning thermosensitivity to wound dressings and drug release [4–8, 18, 21]. PDEAAm is, however, considered to be more suited to biomedical applications than PNIPAAm. It has been reported that the cytotoxicity of PNIPAAm samples was more pronounced than that for PDEAAm ones

when both were prepared in a similar fashion [22]. In this study, chitosan and PDEAAm were used to develop an IPN via photopolymerisation. The PDEAAm-based materials were investigated to establish their potential for easy-removal wound-dressing applications.

Experimental

Materials

Medium molecular weight chitosan, 75–85 % deacetylation (Sigma–Aldrich, St. Louis, MO, USA), N,N-diethylacrylamide (DEAAm, Polysciences Europe, Eppelheim, Germany) and glycerol (SigmaUltra, ≥ 99 %; Sigma–Aldrich) were the main materials used. The UV light-sensitive initiator used was 2-hydroxy-2-methyl-1-phenyl-propanone (Irgacure 2959, Ciba Specialty Chemicals, Basel, Switzerland). Polyethylene glycol 600 dimethacrylate (PEGDMA 600, Sigma–Aldrich) was used as the crosslinking agent. All other reagents (acetic acid, sodium hydroxide, and phosphate-buffered saline) and those used for the Winkler's titration were of analytical grade and used as received.

Preparation of hydrogel film

Chitosan solution was prepared by dispersing 3 g of chitosan powder in 100 ml of aqueous glacial acetic acid (2 %, v/v) under constant stirring at room temperature for about 48 h to achieve dissolution. Known quantities of chitosan solution, DEAAm monomer and glycerol were mixed at different proportions (see Table 1), with the photoinitiator and crosslinker both added at 0.35 % (w/w) with respect to the weight of the DEAAm monomer. The solution was then gently stirred until completely homogeneous and left to stand under vacuum pressure for 24 h to degas the solution and eliminate air bubbles. The mixture was cast onto silicone moulds and subjected to UV curing at room temperature for 20 mins. The Dr. Gröbel UV-Electronik GmbH (Ettlingen, Germany) custom-built irradiation chamber is a controlled radiation source with 20 UV tubes which provide a spectral range of between 315–400 nm. The ability to control the mode of operation of the irradiation chamber provides for three different intensity ranges: high intensity (10–13.5 mW/cm^2), medium intensity (9 mW/cm^2) and low intensity (0.6–4.4 mW/cm^2). The high-intensity range was employed for the polymerisation process. The cured hydrated hydrogel composite films were allowed to dry before neutralising with 1 % aqueous NaOH solution (for any acetic acid present) and further rinsed with distilled water. They were again dried at 37 °C for at least 24 h before testing. The pure chitosan solution was cast on a mould and allowed to dry at 40 °C for at least 24 h.

Table 1 Compositions of the hydrogel film dressings showing the different weight ratios

Sample name	DEAAm %(w/w)	Chitosan solution %(w/w)	Glycerol %(w/w)
DCG 20/70/10	20	70	10
DCG 20/75/5	20	75	5
DCG 20/77.5/2.5	20	77.5	2.5
DCG 30/60/10	30	60	10
DCG 30/65/5	30	65	5
DCG 30/67.5/2.5	30	67.5	2.5

All the samples used for the water uptake and swelling studies, thermal analysis, and oxygen permeation studies were treated with distilled water and dried prior to testing.

Gel fraction analysis

The gel fraction was determined using previously weighed dry chitosan–PDEAAm film samples which were then swollen in excess distilled water for several days to a constant weight (equilibrium swelling) in order to remove the soluble parts. The external solution was replaced with fresh distilled water daily to ensure that the soluble fraction was completely extracted. The equilibrated hydrogel films were then dried in a vacuum oven at 60 °C until they achieved constant weight. The gel fraction was calculated using the equation

$$\text{Gel fraction} (\%) = W_d / W_o \times 100 \qquad (1)$$

where W_d is the dried weight of the sample after extracting the soluble parts, and W_o is the initial weight of the hydrogel after photopolymerisation. Data collected were average values from duplicate samples.

Attenuated total reflectance Fourier transform infrared spectroscopy

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) for the dried films was carried out on a PerkinElmer (Waltham, MA, USA) Spectrum One fitted with a universal ATR sampling accessory. All data were recorded at room temperature in the spectral range 4000–650 cm^{-1}, utilising a 16 scan per sample cycle and a fixed universal compression force.

Thermal analysis of xerogels

Differential scanning calorimetric measurements were carried out with a TA Instruments (New Castle, DE, USA) 2010 DSC. Dried xerogel samples of between 8–12 mg were weighed out and placed in tightly sealed DSC pans. All measurements were conducted in crimped nonhermetic aluminium pans by heating the samples at a rate of 20 °C/min from 20 to 150 °C to remove any thermal history, cooling to −110 °C, and

finally heating again at a rate of 10 °C/min to 300 °C, with an empty crimped aluminium pan being used as the reference cell. The glass transition temperature was defined as the midpoint temperature of the endothermic drift in the heating curves. All DSC tests were carried out in triplicate and under a flow of nitrogen to prevent oxidation. Calibration was previously performed on the machine using indium as standard.

Thermal analysis of hydrated films

A modulated differential scanning calorimeter (MDSC 2920, TA Instruments) coupled with a refrigerated cooling system was used to determine the phase transition temperatures of the chitosan–PDEAAm hydrogels. The samples were pre-equilibrated in water for 24 h; 8–12 mg of sample were crimped in a nonhermetic aluminium pan and heated from 5 to 55 °C at a heating rate of 2 °C/min under a nitrogen atmosphere. An empty sealed pan was used as a reference. The phase transition temperature was defined as the midpoint temperature of the endothermic drift in the heating curves. Calibration was also performed on the machine beforehand using indium as a standard.

Water uptake and swelling studies

Neutralised dried hydrogel films of known weight were immersed in 40-ml phosphate-buffered saline (PBS) solution, pH 7.0, at 37 °C. After known intervals of time had elapsed, the samples were removed, their surfaces carefully blotted with tissue paper to remove excess fluid and they were immediately weighed. After the samples had attained equilibrium swelling, they were transferred to PBS solution at 27 °C. This was carried out to investigate the temperature-dependent behaviour of the dressings. The swelling degree (%) of each hydrogel was calculated using the formula below:

$$\text{Swelling degree} (\%) = (W_t / W_d) \times 100 \qquad (2)$$

where W_t is the weight of the swollen film at a predetermined time, and W_d is the dry weight of the film. Dried pre-weighed samples were also used to study the water

absorption capacities of the hydrogels (water uptake). Water absorption studies are of the utmost importance for applications in wound healing, as they measure the capacity of the material to absorb wound exudates. The samples were placed in PBS solution at room temperature, and their weights were noted periodically after known intervals until they had attained equilibrium. The water uptake was determined using Eq. 3 below. All swelling tests were conducted in triplicate and the mean values were taken. Swelling was plotted as a function of time, and the water content was calculated thus:

$$\text{Water content}(\%) = [(W_t - W_d)/W_t] \times 100. \tag{3}$$

Water retention capacity

To determine the water retention capacities of the hydrogel film dressings, they were taken out of PBS solution after noting their final weights and allowed to equilibrate in air at room temperature. The weights of the samples were recorded periodically as a function of time. The equilibrium water retention (EWR) of the hydrogel was similarly plotted as a function of time using Eq. 2, where W_t is the weight of the film outside the PBS solution after a specific period of time.

Rheometry

Oscillatory parallel-plate rheological measurements were carried out on the swollen hydrogel dressings using an advanced rheometer AR1000 (TA Instruments) fitted with a Peltier device for temperature control. At least four replicate hydrogels were tested at 25 °C using a 20-cm parallel steel plate geometry, and the average values were recorded. A strain sweep was applied from 1.8×10^{-4} to 1.0×10^{-3} at a frequency of 1 Hz, while a weak normal force of 1 ± 0.3 N was exerted on the samples. A low frequency and low strain range was adopted, while all samples were blotted free of water using filter paper prior to testing in an attempt to minimise slippage. Values are reported as the mean±standard deviation of the mean.

Oxygen permeation and measurement

Preparation of reagents

Sodium iodide (NaI) solution (3 M) and sodium hydroxide (NaOH) solution (8 M) were prepared by dissolving 30g NaI in 50 ml of distilled water and 16 g NaOH in 50 ml of distilled water. Both solutions were allowed to cool down and subsequently mixed together. A manganese chloride tetrahydrate ($MnCl_2.4H_2O$) solution was also made up by dissolving 60 g of $MnCl_2.4H_2O$ in 100 ml of distilled water.

0.3567 of dried potassium iodate $K(IO_3)$ was allowed to dissolve in distilled water in a 1-l volumetric flask, which was then filled to the mark to give a standard solution. The molarity was 0.0017 M KIO_3. Sodium thiosulfate pentahydrate ($Na_2S_2O_3.5H_2O$) solution for the titration was prepared by dissolving 0.5 g of its salt in 500 ml of distilled water. This solution was prepared on the day of use and refrigerated because it is perishable and light and temperature sensitive.

Dissolved oxygen measurement

Oxygen penetration through the films was studied by placing and sealing the films on the tops of open 100-ml bottles containing 80 ml of distilled water. The films were held in place with Teflon tape (a modified version of the method of Akturk et al. [23]). The negative control was a closed flask with an airtight cap (preventing oxygen from entering the flask), while the positive control was the open flask (allowing oxygen to enter the flask and dissolve in the water as recipient). The test bottles were placed in an open environment under constant agitation for 24 h. The water samples were then analysed for dissolved oxygen according to Winkler's titration method.

Standardisation of the thiosulfate solution was first done by measuring approximately 50 ml of distilled water into a beaker. Two hundred and fifty microlitres of concentrated sulfuric acid (H_2SO_4), 250 μl $MnCl_2.4H_2O$ solution and 250 μl (NaI 3M+NaOH 8M) solution were added, in that order. Eight millilitres of 0.0017M KIO_3 solution were also added, and the solution was mixed. The solution was titrated against the sodium thiosulfate solution until a pale yellow colour was obtained, before 2–3 drops of starch indicator solution were added. Titration was continued until the first disappearance of the blue colour. The amount of thiosulfate solution added was noted and its molarity (moles per litre) was calculated on the basis of the titrated volume.

The Winkler method for determining dissolved oxygen was carried out by transferring 50 ml of each water sample into a 50 ml bottle, thereby filling it completely. Two hundred and fifty microlitres of $MnCl_2$ solution and 250 μl (NaI 3M+NaOH 8M) solution were added to the 50-ml bottles, which were closed tightly and mixed appropriately, ensuring that no air bubbles were trapped inside the bottles. The precipitates were allowed to settle. Two hundred and fifty microlitres of concentrated H_2SO_4 were added and the bottles were again closed and mixed until all of the precipitate had dissolved, giving a distinctive yellow-gold colour. Fifty millilitres of the solution were transferred to a titration beaker with a glass pipette and titrated against the sodium thiosulfate solution. Titration was continued until a pale yellow colour was observed. Two to three drops of starch indicator solution

were added, and the titration was continued until the first disappearance of the blue colour. The concentration of dissolved oxygen in milligrams per litre (mg/l) was calculated based on the titrated volume of sodium thiosulfate solution. Tests were carried out in duplicate and mean values were reported.

Results and discussion

Film preparation

Blending and/or copolymerisation of chitosan with other polymers has been widely attempted and studied [24–27]. In this case, the DEAAm was polymerised and crosslinked in the presence of chitosan, thereby embedding it within the PDEAAm network, forming an interpenetrating polymer network (IPN). The hydrogel composites formed in this study were white and opaque after curing. All of the samples were easily removed from the silicone moulds and exhibited smooth surfaces. After drying at 38 °C for over 24 h, they became slightly clear and translucent. The hydrogel films, however, became transparent after immersion in aqueous media. Upon handling, it became clear that increasing the glycerol content in the formulation led to increasing flexibility of the hydrogel composite.

Gel fraction

The gel fraction is the mass fraction of the network material resulting from a network-forming polymerisation or crosslinking process. The gel fractions of the chitosan–PDEAAm film wound dressings were all above 60 %. The chitosan–PDEAAm films that contained 30 % (w/w) DEAAm had marginally higher gel fractions, consistent with the slightly higher crosslinker content and also increased photoinitiator content. Increasing the photoinitiator content increases the amount of free radicals generated during UV irradiation, hence improving the crosslinking density and the formation of a stable hydrogel network [26]. High chitosan contents also appeared to have a negative effect on the gel fraction of the films, since the higher the chitosan content, the lower the gel fraction. This could be due to the highly aqueous state of the chitosan solution, which inhibits the crosslinking reaction in the PDEAAm polymer and the overall film network. Similar observations have been reported in the literature [2]. It could be inferred that a combination of unreacted and uncrosslinked DEAAm monomers and some of the chitosan and glycerol that were not properly interlaced within the interpenetrating network were extracted into the solution over the time period. Table 2 shows the gel fraction data for the films and their compositions.

ATR-FTIR analysis

FTIR analysis was carried out on the dry chitosan–PDEAAm hydrogel wound dressings. A comparison of the spectra of chitosan, PDEAAm, and chitosan–PDEAAm is shown in Figs. 1 and 2. The characteristic peaks of chitosan due to OH/NH$_2$ stretching, C=O (amide 1) stretching and NH$_2$ (amide II) bending are located at 3253 cm^{-1}, 1685 cm^{-1} and 1560 cm^{-1}, respectively [25, 27]. The strong absorption band between 800 and 1200 cm^{-1} is characteristic of the pyranose rings of chitosan [28, 29]. The peaks at 1153, 1054, 1023 and 852 cm^{-1} correspond to stretching bands of the bridging oxygen (C–O–C) [30]. Moreover, the spectrum of chitosan can be influenced by parameters such as the deacetylation percentage or the crystallinity [31].

All of the spectra for the chitosan–PDEAAm films exhibit two characteristic strong bands from amide groups at 1640–1619 and 1560 cm^{-1}, ascribed to the characteristic absorption of C=O stretching and NH$_2$ bending modes, respectively. The bands discernible at 1431/1380/1271 cm^{-1} have previously been reported to be associated with symmetrical C–H bending in PDEAAm [32]. The broad band that corresponds to the O–H stretching vibration (3400–3100 cm^{-1}) was more intense due to the incorporation of glycerol and PDEAAm. The incorporation of glycerol into polymer networks can lead to higher levels of O–H bonding and consequently broader band areas [33] due to the hydroxyl group of the glycerol and entrapped moisture. This is due to the fact that glycerol, due to its hydrophilic nature, retains water in the film matrix. PDEAAm is also hydrophilic at room temperature, and hence can also absorb moisture from the atmosphere. All of the chitosan–PDEAAm samples, without exception, showed a very strong and large peak at around 1029–1024 cm^{-1}, confirming the presence of chitosan in the network.

The amide II absorption band at 1560 cm^{-1} was very strong and distinct in samples DCG 20/77.5/2.5 and DCG 30/67.5/2.5. This shoulder at 1560 cm^{-1}, which grew smaller and less distinct in the sample spectrum as the glycerol content in the composition increased, has also been assigned to –NH$_3^+$ $^-$OOCCH$_3$ interactions [34]. A similar phenomenon had previously been observed by Lavorgna et al. [35], where they reported that the shoulder disappears when glycerol is added as plasticiser to a chitosan–clay nanocomposite film formulation. In this case, though, the amide II absorption band at 1560 cm^{-1} would have mostly come from the PDEAAm, and the interaction should occur between the amide of the PDEAAm and the glycerol, due to the higher PDEAAm content in the IPNs.

Conversely, the C=O amide I band at 1638 cm^{-1} grew larger while undergoing a displacement in its position to lower wavelengths: to 1627 and 1619 cm^{-1} in DCG 20/75/5 and DCG 20/70/10, respectively. DCG 20/77.5/2.5 exhibited a strong band at 1151 cm^{-1}, which was less visible in DCG

Table 2 Gel fraction data, glass transition temperatures, and average phase transition values for chitosan–PDEAAm wound dressings with different compositions

Samples	Gel fraction (%)	Glass transition temperature (°C)	Phase transition temperature (°C)
DCG 20/77.5/2.5	61.5	175	28.7
DCG 20/75/5	62.9	182	29.3
DCG 20/70/10	63.6	169	28.5
DCG 30/67.5/2.5	61.4	150	28.6
DCG 30/65/5	67.2	183	28.9
DCG 30/60/10	67.6	170	29.4

20/75/5 and much less so in DCG 20/70/10, consistent with the decrease in chitosan concentration. The peak at 1217 cm^{-1} grew larger as the glycerol content increased. Similar observations were made in the films with 30 % (w/w) DEAAm.

DCG 30/60/10 had the most intense absorption band at 3290 cm^{-1} (for all the chitosan–PDEAAm films), followed by 5 % (w/w) and 2.5 % (w/w) glycerol, in that order, for the films with 30 % (w/w) DEAAm (confirming the higher intensity of the O–H bonds). Again, the absorption peak at 1151 cm^{-1} is clearly visible in DCG 30/67.5/2.5, and slightly so in DCG 30/65/5, but disappears almost completely in DCG 30/60/10.

Thermal analysis of xerogels

The thermal behaviour of each dried chitosan–PDEAAm hydrogel film sample was studied in duplicate. The thermal properties of pure chitosan film and chitosan–PDEAAm were difficult to determine, mainly due to the problems associated with their hygroscopic nature. Both chitosan and the plasticiser (glycerol) have the ability to absorb moisture, leading to the creation of hydrogen bonds [36], which strongly affects the film's thermal and mechanical properties, resulting in a substantial drop in the glass transition temperature [37]. Moisture content affects the T_g values of the films; in fact, water also acts as a plasticiser by increasing the molecular mobility of chitosan films, hence lowering T_g values. A considerable number of papers dealing with the influence of water on the relaxation properties of chitosan have been published in recent years [33, 38, 39]. Such a characteristic explains the fact that so many different values of T_g for plasticised and unplasticised chitosan have been reported, with differences that can sometimes be as large as 50 °C. Sakurai et al. [40] reported that the T_g of chitosan, determined by DSC and
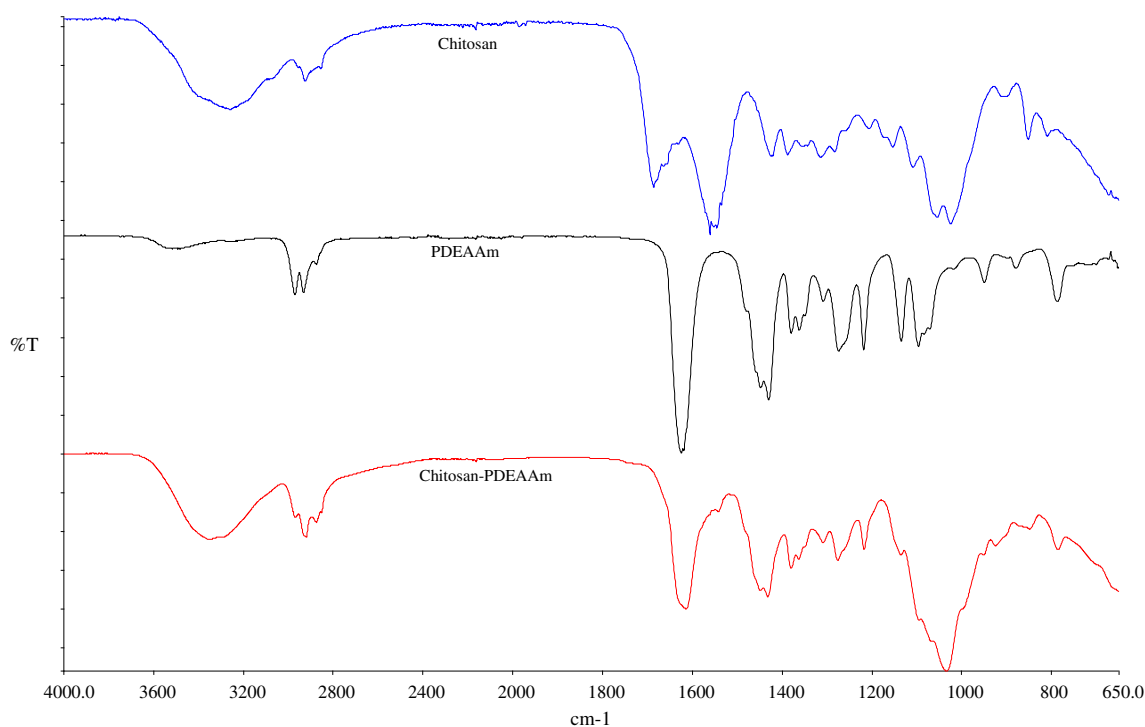
Fig. 1 FTIR spectra of chitosan, PDEAAm, and the chitosan–PDEAAm (DCG 30-60-10) hydrogel wound dressing
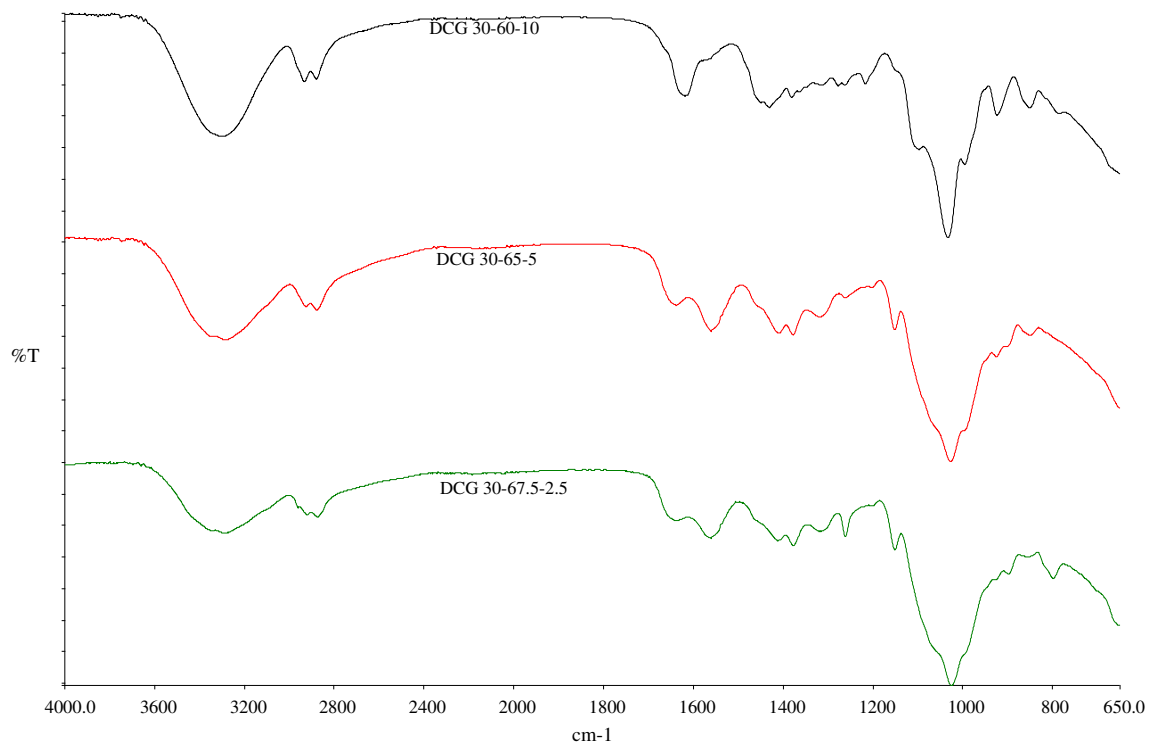
Fig. 2 FTIR spectra of chitosan–PDEAAm hydrogel wound dressings with different compositions

dynamic mechanical analysis (DMA), was 203 °C. Silva et al. [41] found a glass transition at 243 °C, Shanta and Harding [42] observed a sharp glass transition at 195 °C, and Dong et al. [43] reported the T_g of chitosan to be around 140 °C after investigating using four different characterisation techniques. In an attempt to prevent any such variation in T_g values for the chitosan–PDEAAm hydrogels, all samples were pre-conditioned at about 50 °C for 48 h prior to analysis.

Glycerol, due its plasticising effect, decreases the glass transition temperature of polysaccharide films, which is in agreement with the free volume theory of plasticisation. Suyatma et al. [44] reported the T_g of unplasticised chitosan film to be around 196 °C, which reduced to approximately 184, 178 and 169 °C for the plasticised films containing 5, 20 and 40 % glycerol, respectively. The increase in glycerol concentration led to an increase in the free volume and mobility of the molecules, changing the physical structure of the chitosan film and thus leading to a decrease in the T_g value. The glycerol contained in the film dressing results in a more hygroscopic matrix. It is worth noting that the hydrophilic glycerol is known to entrap additional water in the network, even in a "dried" state. Glycerol changes the polymer network, creating mobile regions with greater interchain distances, promoting water clustering [45, 46], and thus increasing the moisture content in the films.

The T_g of PDEAAm has been reported to be 86 °C [14] and 90 °C [47]. The miscibility of the components in the network allowed for a single T_g to be observed in all cases (see Table 2). Also, due to the high chitosan content in the network, its glass transition seemed to dominate that of PDEAAm, although the T_g value for the chitosan–PDEAAm was less than that of pure chitosan in all cases. The average glass transition values of all the compositions were in the range 150–182 °C. The T_g values obtained here are in agreement with those reported in other works for chitosan and glycerol-plasticised chitosan [33, 40, 44].

Thermal analysis of hydrated samples

DSC scans (such as that illustrated in Fig. 3) showed that the hydrogel dressings displayed phase transition behaviour. PDEAAm is a typical thermosensitive polymer, with a phase transition temperature reported to be around 28–32 °C [14, 48, 49]. The transition in the heating curves appeared between 28 °C and 30 °C for all of the samples. This confirms that the reversible phase transition of the PDEAAm was retained in the hydrogel film network. Chen et al. [8] also reported retention of the LCST of PNIPAAm in a wound dressing consisting of polypropylene nonwoven fabric grafted with PNIPAAm and chitosan. There did not appear to be any observable trend or effect of film composition on the phase transition temperature, so it is assumed the chitosan and plasticiser (glycerol) content did not have any discernible effect on the thermosensitivity of the network.
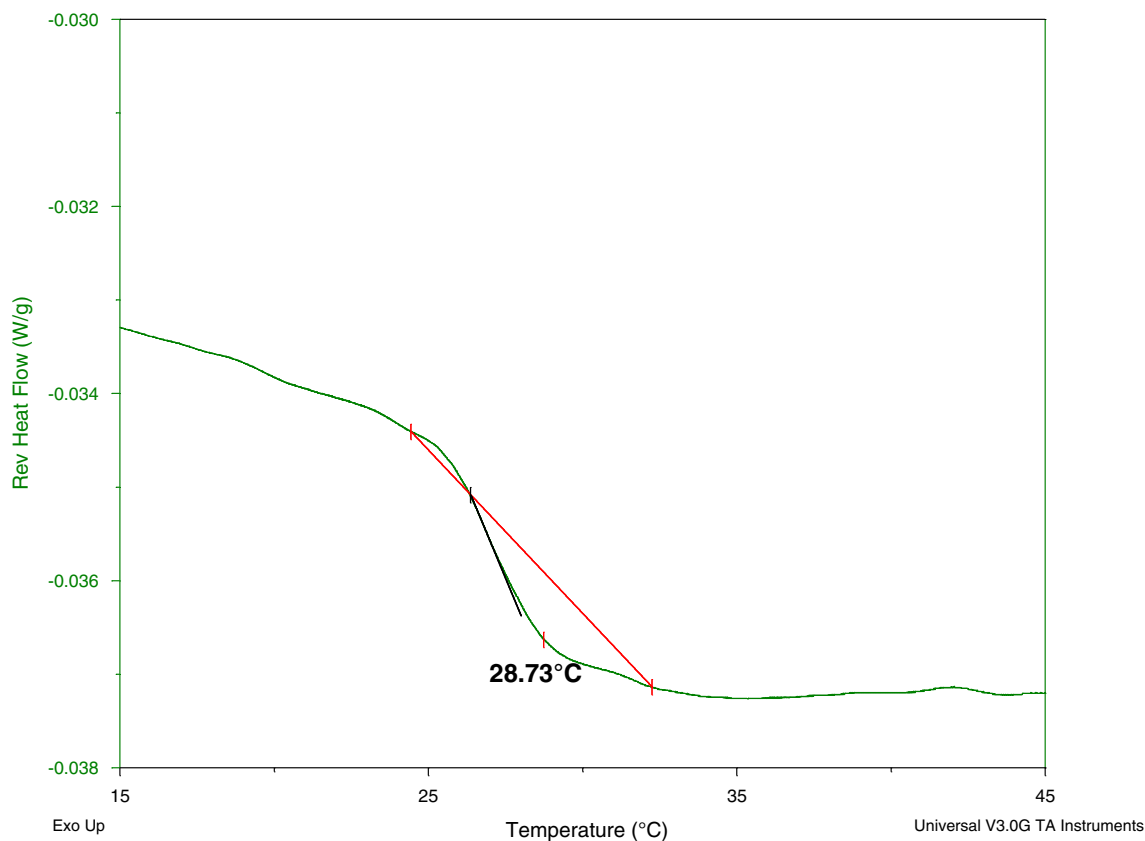
Fig. 3 A representative thermal scan of the PDEAAm–chitosan (DCG 20/77.5/2.5) hydrogel wound dressing

In an aqueous environment below its LCST, the chitosan–PDEAAm film undergoes a phase transition, absorbs water and becomes a swollen polymer. Above the LCST, the hydrogel collapses to a state with a much lower water content. This property change potentially makes the removal of the dressing from an injured surface much easier when it is wetted with cold water, thereby avoiding further trauma to healing tissue and reducing the accompanying pain. Figure 4 gives a clear illustration of this phenomenon. Table 2 shows the average transition temperatures for all of the chitosan–PDEAAm hydrogel dressings.

The step change is an endothermic peak and is not sharp because the phase transition for PDEAAm polymer and copolymers is not a dramatic one, as would be observed in PNIPAAm polymer, for instance.

Swelling and water-uptake studies

As far as surface characteristics are concerned, the hydrophilicity of materials has been shown to be an important factor for wound dressings. A water-uptake study is a gravimetric test which determines the maximum amount absorbed and retained by the dressing as a percentage. This test is designed to determine how a dressing performs under more extreme conditions than they are likely to encounter in vivo. Essentially, the increase in weight of the dressing after absorbing fluid and swelling over a given time period is measured and used as an indication of the water uptake and retention.

Figure 4 shows the temperature-dependent swelling profile of the chitosan-PDEAAm hydrogel dressings at 37 °C and subsequently at 27 °C. The samples were clear and transparent after immersion in solution, with the exception of DCG 20/77.5/2.5, possibly due to its high chitosan content. Upon reducing the temperature of the swelling medium, the hydrophilicity of the network increased due to the thermosensitive character of the incorporated PDEAAm. At 27 °C, below the VPTT (volume phase transition temperature), hydrophilic interactions in the PDEAAm become more dominant, leading to increased water sorption and swelling. Conversely, at 37 °C (above the transition temperature of PDEAAm), the amide–water hydrogen bonds are disrupted and the hydrophobic interactions due to the hydrocarbon backbone and the side-chain alkyl groups in the DEAAm become dominant, but the hydrogel still absorbs water and swells, which to a large extent is due to the presence of glycerol, a hydrophilic polyol, as well as the hydrophilic groups in chitosan (hydroxyl

Fig. 4 Swelling profiles of the hydrogel wound dressings with different compositions at 37 °C and 27 °C
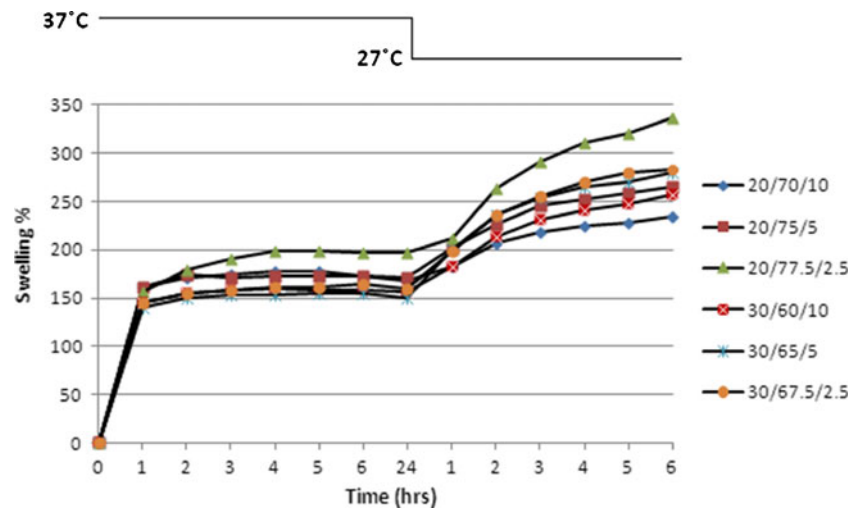
and amino groups). The PDEAAm in the film network largely controls and determines the water uptake behaviour of the hydrogel dressings. This thermodependent water-uptake behaviour is a unique property that suggests the chitosan–PDEAAm hydrogel wound dressing would be beneficial for the alleviation of pain during dressing changes and removal. Thermodependent hydrogel swelling has been reported in other studies and proposed for potential wound-dressing applications [5, 7, 8].

In order to determine the water-absorption capacity or fluid uptake of the different hydrogel dressing compositions, their swelling profiles were studied in PBS at 37 °C and also at room temperature. At 37 °C, the samples took about 3–4 h to reach equilibrium. At a test temperature of 37 °C, DCG 20/77.5/2.5 had the highest water sorption compared to all the other samples, while DCG 30/65/5 had the lowest. Also, at room temperature, the same film dressing with composition 20/77.5/2.5 again had the highest swelling percentage.

At 37 °C, the swelling kinetics were more affected by the DEAAm content (as it is the thermosensitive component, and the test temperature was above its critical temperature), since the compositions with less DEAAm (20 % w/w) absorbed more water than those with a higher concentration (30 % w/w) (see Fig. 4). The higher the DEAAm content in the sample, the greater the hydrophobic interactions in the overall film network, due to the available alkyl side chains and the effect of the test temperature. On the other hand, the glycerol content had a more significant effect on the swelling ratios at room temperature (Fig. 5). With a higher glycerol concentration, less of the solution was absorbed by the hydrogel, irrespective of the DEAAm content. Under room-temperature swelling conditions (20 °C), the DEAAm concentration in the hydrogel film network had little effect. Increased glycerol content is likely to have resulted in films with greater dimensional stability. This

behaviour is most probably due to the formation of a more compact crosslink network induced by hydrogen bonds between the chitosan and glycerol. Similar effects on the swelling of chitosan films due to glycerol content have been reported previously [32]. DCG 20/77.5/2.5 reached an equilibrium swelling ratio of about 560 %, a little higher than the swelling ratio of 500 % reported for a thiolated chitosan with a poly(*N*-isopropylacrylamide) wound dressing by Radhakumary et al. [7]. Essentially, all of the chitosan–PDEAAm films attained maximum equilibrium water contents of 66 % and above, with DCG 20/77.5/2.5 achieving a water content of over 82 % (Table 3). The commercial Luofocon® hydrogel dressing has a moisture content of approximately 80 %. This relatively high fluid uptake for DCG 20/77.5/2.5 means that it has the potential to prevent the accumulation of wound fluids (which could lead to maceration) by adsorption of the exudate. Interestingly, at both temperatures under study, all of the samples seemed to reach equilibrium in PBS solution at about the same time period: between three and four hours at 37 °C and around 120 h after swelling at ambient temperature.

Following swelling for approximately 24 h, the samples were removed from solution and left in air at room temperature. Water loss from the samples tended to be linear, with similar slopes (increasing linearly with time), which would indicate that all of the hydrogel films had similar water retention capabilities regardless of their compositions. DCG 20/77.5/2.5 showed the highest equilibrium water retention (EWR), with a water content of 55.7 % after a period of 4 h. This is due to the initial high fluid absorption capacity of the sample, so it will take much longer to lose its water content. Witthayaprapakorn [50] reported an EWR of 22±2 % over the same period for photopolymerised Na-AMPS-based (sodium salt of 2-acrylamido-2-methylpropane sulfonic acid) wound dressings.

Fig. 5 Swelling profiles of hydrogel dressings immersed in PBS solution at room temperature
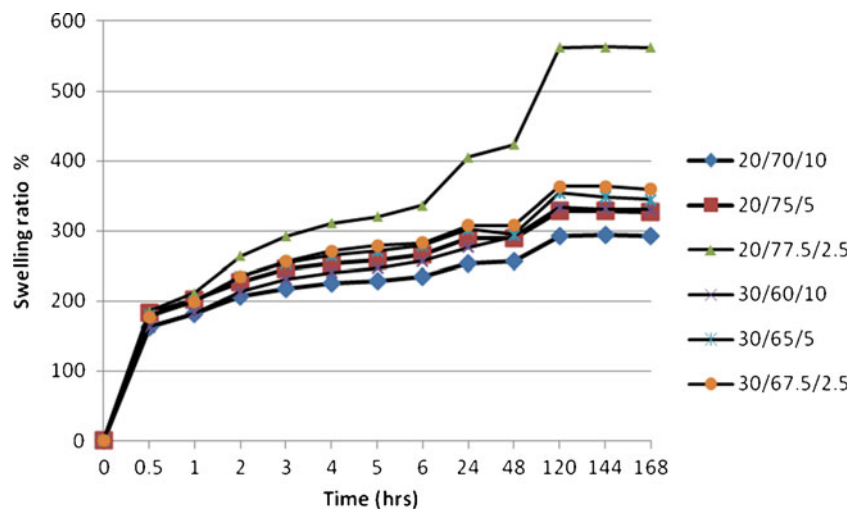
It is clear from these studies that the films will decrease in water content when exposed to air under dry conditions over long periods. Thus, these dressings will be more beneficial to wounds with moderate exudates rather than to dry wounds. This water loss could enable the films to take up exudates and oedema fluid from the wounds into the dressing by an active upward-directed process when used in exudating wounds, as has been reported for some commercially available dressings [51]. However, it is not unusual for dressings to be kept moist if desired by spraying saline or water, since these chitosan–PDEAAm hydrogel films rapidly imbibe water at ambient temperature due to their temperature-dependent character.

Rheometry

The storage modulus (G') values for the tested samples are given in Fig. 6. The nature of the film network constituents and the crosslinking agent concentration had obvious effects on the mechanical properties of the hydrogels. Firstly, it was evident that the storage modulus was influenced by the crosslinker concentration and the chitosan content. The

Table 3 Profiles of the hydrogel dressings immersed in PBS solution, showing their percentage equilibrium water contents at room temperature

Sample	Equilibrium water content
DCG 20/77.5/2.5	82.2 %
DCG 20/75/5	69.5 %
DCG 20/70/10	66.0 %
DCG 30/67.5/2.5	72.6 %
DCG 30/65/5	71.7 %
DCG 30/60/10	70 %

samples with 30 % (w/w) DEAAm content had a higher concentration of crosslinker due to the fact that the crosslinker content employed was added with respect to the DEAAm content. Hence, the samples with 30 % (w/w) DEAAm concentration exhibited higher storage modulus values than those with 20 % (w/w). The effect of chitosan concentration is also apparent (DCG 30/65/5, 18,730±3144 Pa and DCG 30/67.5/2.5, 19,060±2,284 Pa); increasing as the chitosan content increased and the glycerol content decreased.
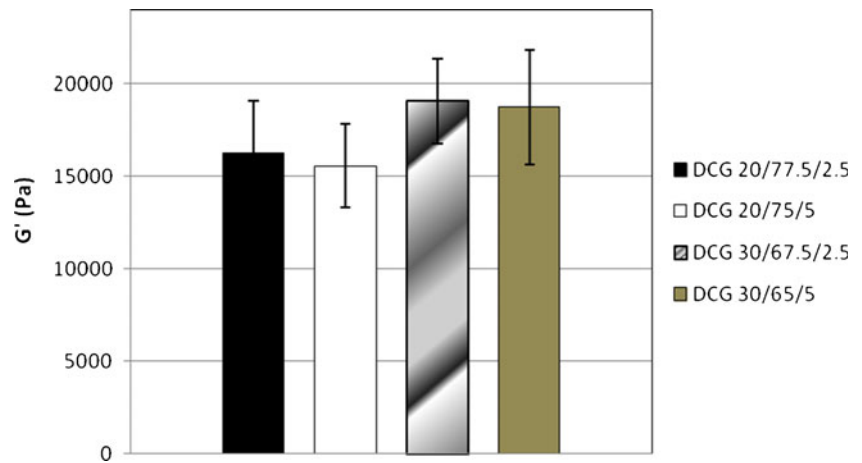
Oxygen penetration

The Winkler method is a technique that uses titration to measure dissolved oxygen in water samples. The chemical determination of oxygen concentrations in water is based on the method first proposed by Winkler [52] and modified by Strickland and Parsons [53]. This procedure describes a method for the determination of dissolved oxygen in aqueous samples, expressed as mgO_2/l. Table 4 gives the oxygen permeabilities of the films as a function of the amount of dissolved oxygen in the distilled water samples.

The negative control had significantly lower dissolved oxygen (DO) values than the others. Although none of the film wound-dressing groups reached DO values that were as high as the open (positive) control, oxygen penetration was not very different among the chitosan–PDEAAm films. However, there appeared to be an increase in oxygen penetration as the plasticiser content in the films increased. This may be due to the hydrophilicity of glycerol, which attracts moisture from the atmosphere, hence increasing permeability. Wittaya-areekul and Prahsarn [54] have previously reported an increase in oxygen penetration in polysaccharide composite films due to increase in the concentration of propylene glycol, a hydrophilic plasticiser. Silver [55] and

Fig. 6 Representative average storage modulus values of all the PDEAAm–chitosan hydrogel dressings. *Average of at least 4 replicates ± standard deviation

Winter [56] investigated the effects of oxygen availability on wound healing, and the results of the various experiments reported strongly suggested that the epidermal cells around a wound surface can utilise more oxygen if it is made available by switching from anaerobic to aerobic metabolism of carbohydrates, which results in more rapid epidermal regeneration. Pandit and Faldman [57] also reported that oxygen treatment appeared to enhance the healing response significantly. The wounds covered with the oxygen-impermeable dressings were significantly better than those with the oxygen-permeable dressings in the oxygen-treated group after 1 week, but the wounds covered with the oxygen-permeable dressings had healed better at 3 weeks. Therefore, they proposed that oxygen-impermeable dressings may be useful only in the early stages of healing, before granulation tissue formation. There also is recent evidence that keratinocyte motility is enhanced by low oxygen tension. It is possible that early hypoxia has a stimulatory effect on the healing process, whereas the persistence of low oxygen tension might actually impair wound repair and tissue integrity. This suggests that nature can be

Table 4 Oxygen permeabilities of hydrogel film dressings with different compositions

Sample	Oxygen permeability (amount of dissolved oxygen, mg/l)
Negative control	8.74±0.16
Positive control	9.68±0.12
DCG 20/77.5/2.5	9.06±0.04
DCG 20/75/5	9.05±0.07
DCG 20/70/10	9.08±0.08
DCG 30/67.5/2.5	9.08±0.10
DCG 30/65/5	9.12±0.11
DCG 30/60/10	9.18±0.06

Each value is the average of duplicate samples ± standard deviation

improved upon by using dressings that prevent scab formation and are oxygen permeable.

Conclusion

The novel thermoresponsive chitosan–PDEAAm films exhibited adequate mechanical properties to warrant further investigation for use as wound/burn dressings, and were found to switch from a slightly hydrophilic to a highly hydrophilic surface upon being exposed to a solution at a lower temperature. This thermosensitive character makes the films easily detachable, enabling frequent dressing changes if desired, or removal without causing further injury to healing tissues. The thermosensitivity of PDEAAm was retained in the film composite network, as illustrated by the MDSC and swelling studies. The results showed that the DCG 20/77.5/2.5 chitosan–PDEAAm film had the best potential to be employed as a moist wound dressing due to its high water content compared to the other compositions. This means that it has the capacity to retain a moist environment over a wound for a much longer period, thereby facilitating the healing process. A cold pack can be placed over the film prior to dressing removal, allowing it to swell and detach easily from a wound site, thus helping to prevent the secondary injury usually associated with dressing changes/removal. The ease of removal of the chitosan–PDEAAm dressing from a wound site due to its switchable hydrophilicity further enhances its potential for application. All of the dressings were also found to be oxygen permeable.

Peel adhesion tests, drug (antibiotic) incorporation, drug release and its inhibitory effect on bacterial viability, and an in vitro cytotoxicity study to ascertain the effects of the wound dressing on skin fibroblasts would constitute the next phase of the study. All of these will be done to evaluate the potential of this chitosan–PDEAAm hydrogel system to effectively interact with—and protect wounds from—local infection, while providing a good moist healing environment with the release of antimicrobial drugs in a controlled manner. This is with a

view to developing an ideal novel dressing with reduced adhesiveness (to facilitate painless removal from the wound site) as a result of its thermosensitivity, antibacterial properties, and with considerable potential to be used as a wound dressing, especially for burns and donor sites.

References

1. Murakami K, Aoki H, Nakamura S, Nakamura S-I, Takikawa M, Hanzawa M, Kishimoto S et al (2010) Hydrogel blends of chitin/chitosan, fucoidan and alginate as healing-impaired wound dressings. Biomaterials 31:83–90

2. Sung JH, Hwang MR, Kim JO, Lee JH, Kim YI, Kim JH, Chang SW, Jin SG, Kim JA, Lyoo WS, Han SS, Ku SK, Yong CS, Choi HG (2010) Gel characterisation and in vivo evaluation of minocycline-loaded wound dressing with enhanced wound healing using polyvinyl alcohol and chitosan. Int J Pharm 392:232–240

3. Hollinworth H, Collier M (2000) Nurses' views about pain and trauma at dressing changes: results of a national survey. J Wound Care 9(8):369–373

4. Lin S-Y, Chen K-S, Chu L-R (2001) Design and evaluation of drug-loaded wound dressing having thermoresponsive, adhesive, absorptive and easy peeling properties. Biomaterials 22:2999–3004

5. Chen K-S, Ku Y-A, Lee C-H, Lin H-R, Lin F-H, Chen T-M (2005) Immobilisation of chitosan gel with cross-linking reagent on PNIPAAm gel/PP nonwoven composites surface. Mater Sci Eng C 25:472–478

6. Yang JM, Yang SJ, Lin HT, Wu T-H, Chen H-J (2008) Chitosan containing PU/Poly(NIPAAm) thermosensitive membrane for wound dressing. Mater Sci Eng C 28:150–156

7. Radhakumary C, Antonty M, Sreenivasan K (2011) Drug loaded thermoresponsive and cyto-compatible chitosan based hydrogel as a potential wound dressing. Carbohydr Polym 83:705–713

8. Chen J-P, Kuo C-Y, Lee W-L (2012) Thermoresponsive wound dressings by grafting chitosan and poly(N-isopropylacrylamide) to plasma-induced graft polymerization modified non-woven fabrics. Appl Surf Sci. doi:10.1016/j.apsusc.2012.02.106

9. Kim J, Cai Z, Lee HS, Choi GS, Lee DH, Jo C (2011) Preparation and characterization of a bacterial cellulose/chitosan composite for potential biomedical application. J Polym Res 18(4):739–744

10. Mourya VK, Inamdar NN (2008) Chitosan modifications and applications: opportunities galore. React Funct Polym 68:1013–1051

11. Muzzarelli RAA (2009) Chitins and chitosans for the repair of wounded skin, nerve, cartilage and bone. Carbohydr Polym 76:167–182

12. Jayakumar R, Prabaharan M, Kumar PT, Sudheesh NSV, Tamura H (2011) Biomaterials based on chitin and chitosan in wound dressing applications. Biotechnol Adv 29:322–337. doi:10.1016/j.biotechadv.2011.01.005

13. Shi CM, Zhu Y, Ran XZ, Wang M, Su Y, Cheng TM (2006) Therapeutic potential of chitosan and its derivatives in regenerative medicine. J Surg Res 133:185–192

14. Ngadaonye JI, Geever LM, Cloonan MO, Higginbotham CL (2012) Photopolymerised thermo-responsive poly(N,N-diethylacrylamide)-based copolymer hydrogels for potential drug delivery applications. J Polym Res 19:9822

15. Okano T, Bae YH, Jacobs H, Kim SW (1990) Thermally on-off switching polymers for drug permeation and release. J Controlled Release 11:255–265

16. Okuyama Y, Yoshida R, Sakai K, Okano T, Sakurai Y (1993) Swelling controlled zero order and sigmoidal drug release from thermo-responsive poly(N-isopropylacrylamide-co-butyl methacrylate) hydrogel. J Biomater Sci Polym Ed 4:545–556

17. Kim SJ, Lee CK, Lee YM, Kim SI (2003) Preparation and characterisation of thermosensitive poly(N-isopropylacrylamide)/poly(ethylene oxide) semi-interpenetrating networks. J Appl Polym Sci 90:3032–3036

18. Zhang JT, Huang SW, Liu J, Zhuo RX (2004) Temperature sensitive poly(N-isopropyl acrylamide)/poly(N-isopropylacrylamide) interpenetrating polymer networks for drug delivery. J Polym Sci Polym Chem 42:1249–1254

19. Deshpande DS, Bajpai R, Bajpai AK (2012) Synthesis and characterization of polyvinyl alcohol based semi interpenetrating polymeric networks. J Polym Res 19:9938

20. Yun J, Im JS, Kim HI, Lee YS (2012) Effect of oxyfluorination of PVA/PNIPAAm hydrogel on temperature responsive drug release. J Polym Res 19:9887

21. Yang JM, Lin HT (2004) Properties of chitosan containing PP-g-AA-g-NIPAAm bigraft nonwoven fabric for wound dressing. J Membr Sci 243:1–7

22. Panayiotou M, Pöhner C, Vandevyver C, Wandrey C, Hilbrig F, Freitag R (2007) Synthesis and characterisation of thermoresponsive poly(N,N-diethylacrylamide) microgels. React Funct Polym 67:807–819

23. Akturk O, Tezcaner A, Bilgili H, Deveci MS, Gecit MR, Keskin D (2011) Evaluation of sericin/collagen membranes as prospective wound dressing biomaterial. J Biosci Bioeng 112:279–288

24. Sakchai W, Chureerat P, Srisagul S (2006) Development and in vitro evaluation of chitosan- eudragit RS 300 composite wound dressings. AAPS Pharm Sci Tech 7:E1–E6

25. Ganji F, Abdekhodaie MJ (2010) Chitosan-g-PLGA copolymer as a thermosensitive membrane. Carbohydr Polym 80:740–746

26. Yang C, Xu L, Zhou Y, Zang X, Huang X, Wang M, Han Y, Zhai M, We S, Li J (2010) A green fabrication approach of gelatin/CM-chitosan hybrid hydrogel for wound healing. Carbohydrate Polym 82:1297–1305

27. Abugoch LE, Tapia C, Villamán MC, Yazdani-Pedram M, Díaz-Dosque M (2011) Characterisation of quinoa protein–chitosan blend edible film. Food Hydrocolloids 25:879–886

28. Brugnerotto J, Lizardi J, Goycoolea FM, Arguelles-Monal W, Desbrieres J, Rinaudo M (2001) An infrared investigation in relation with chitin and chitosan characterisation. Polymer 42(8):3569–3580

29. Prashanth KVH, Kittur FS, Tharanathan RN (2002) Solid state structure of chitosan prepared under different N-deacetylating conditions. Carbohydrate Polym 50(1):27–33

30. Brandenburg K, Seydel U (1996) Fourier transform infrared spectroscopy of cell surface polysaccharides. Wiley–Liss, New York

31. Lima CGA, de Oliveira RS, Figueiro SD, Wehmann CF, Goes JC, Sombra ASB (2006) DC conductivity and dielectric permittivity of collagen–chitosan films. Mater Chem Phys 99:284–288

32. Chen J, Liu M, Liu H, Ma L (2009) Synthesis, swelling and drug release behaviour of poly(N,N-diethylacrylamide-co-N-hydroxymethyl acrylamide) hydrogel. Mater Sci Eng C 29:2116–2123

33. Cerqueira M, Souza B, Teixeira J, Vicente A (2011) Effect of glycerol and corn oil on physico-chemical properties of polysaccharide films—a comparative study. Food Hydrocoll 27:175–184

34. Brown CD, Kreilgaard L, Nakakura M, Caram-Lelham N, Pettitb DK, Gombotzb WR et al (2001) Release of PEGylated granulocyte–macrophage colony stimulating factor from chitosan/glycerol films. J Controlled Release 72:35–46

35. Lavorgna M, Piscitelli F, Mangiacapra P, Buonocore GG (2010) Study of the combined effect of both clay and glycerol plasticiser on the properties of chitosan films. Carbohydr Polym 82:291–298

36. Mucha M, Pawlak A (2005) Thermal analysis of chitosan and its blends. Thermochima Acta 427:69–76

37. Lazaridou A, Biliaderis CG (2002) Thermophysical properties of chitosan, chitosan–starch and chitosan–pellulan films near the glass transition. Carbohydr Polym 48:179–190

38. Hasegawa M, Isogai F, Onabe F, Usuda M, Atalla RH (1992) Characterisation of cellulose–chitosan blend films. J Appl Polym Sci 45:1873–1879

39. Czihak C, Muller M, Schober H, Heux L, Vogl G (1999) Dynamics of water adsorbed to cellulose. Physica B 266:87–91

40. Sakurai K, Maegawa T, Takahashi T (2000) Glass transition temperature of chitosan and miscibility of chitosan/poly(N-vinyl pyrrolidone) blends. Polymer 41:7051–7056

41. Silva CL, Pereira JC, Ramalho A, Pais ACC, Sousa JJS (2008) Films based on chitosan polyelectrolyte complexes for skin delivery: Development and characterisation. J Membr Sci 320:268–279

42. Shanta K, Harding DRK (2002) Synthesis and characterisation of chemically modified chitosan microspheres. Carbohydr Polym 48:247–253

43. Dong Y, Ruan Y, Wang H, Zhao Y, Bi D (2004) Studies on glass transition temperature of chitosan with four techniques. J Appl Polym Sci 93:1553–1558

44. Suyatma NE, Tighzert L, Copinet A, Coma V (2005) Effects of hydrophilic plasticisers on mechanical, thermal, and surface properties of chitosan films. J Agric Food Chem 53:3950–3957

45. Diab T, Biliaderis CG, Gerasopoulos D, Sfakiotakis E (2001) Physicochemical properties and application of pullulan edible films and coatings in fruit preservation. J Sci Food Agric 81:988–1000

46. Olivas GI, Barbosa-Cánovas GV (2008) Alginate–calcium films: water vapour permeability and mechanical properties as affected by plasticiser and relative humidity. LWT—Food Sci Technol 41:359–366

47. Silva MESR, Dutra ER, Mano V, Machado JC (2000) Polym Degrad Stab 67:491–495

48. Idziak I, Avoce D, Lessard D, Gravel D, Zhu XX (1999) Thermosensitivity of aqueous solutions of poly(N,N-diethylacrylamide). Macromolecules 32:1260–1263

49. Liu H, Liu M, Ma L, Chen J (2009) Thermo- and pH-sensitive comb-type grafted poly(N,N-diethylacrylamide-co-acrylic acid) hydrogels with rapid response behaviours. Eur Polym J 45:2060–2067

50. Witthayaprapakorn C (2011) Design and preparation of synthetic hydrogels via photo-polymerisation for biomedical use as wound dressings. Procedia Eng 8:286–291

51. Kichofen B, Wokalek H, Scheel D, Ruh H (1986) Chemical and physical properties of a hydrogel wound dressing. Biomaterials 7:67–72

52. Winkler LW (1888) Die Bestimmung des in Wasser gelösten Sauerstoffen. Be Dtsch Chem Ges 21:2843–2855

53. Strickland JDH, Parsons TR (1968) Determination of dissolved oxygen. A practical handbook of seawater analysis. Bull Fisheries Res Board Canada 167:71–75

54. Wittaya-areekul S, Prahsarn C (2006) Development and in vitro evaluation of chitosan polysaccharides composite wound dressings. Int J Pharm 313:123–128

55. Silver IA (1972) Oxygen tension and epithelialisation. In: Maibach HI, Rovee DT (eds) Epidermal wound healing. Year Book Medical, Chicago, p 291

56. Winter GD (1972) Epidermal regeneration studied in the domestic pig. In: Maibach HI, Rovee DT (eds) Epidermal wound healing. Year Book Medical, Chicago, p 71

57. Pandit AS, Faldman DS (1994) Effect of oxygen treatment and dressing oxygen permeability on wound healing. Wound Repair Regen 2(2):130–137