



Semi-solid poly(vinyl alcohol) hydrogels containing ginger essential oil encapsulated in chitosan nanoparticles for use in wound management

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Abstract

Encapsulation of ginger (*Zingiber officinale* Roscoe) essential oil (GEO) in different amounts (i.e. 1%, 3%, and 5% v/v) within chitosan nanoparticles (CNPs) was studied. It was done by a two-step method: oil in water emulsion (O/W) and ionic gelation using sodium tripolyphosphate (STPP) as the crosslinking agent. GEO-loaded CNPs appeared to be more aggregated with an increase in the GEO content. An increase in the GEO content caused %encapsulation efficiency (EE) to decrease, whereas %loading capacity (LC) to increase. Semi-solid PVA hydrogels containing different amounts (i.e. 0.5%, 1.0%, and 1.5% w/v) of the GEO-loaded CNPs were prepared and characterized for their mechanical properties, water content, and release characteristics. Hardness, compressibility, and water content of the as-prepared hydrogels decreased with an increase in the amount of the GEO-loaded CNPs, while the amount of GEO released from the hydrogels was found to increase. Notwithstanding, incorporation of GEO-loaded CNPs in the hydrogels sustained the release of GEO. As these semi-solid hydrogels appeared to be non-toxic to both NCTC clone 929 and NHDF cells, they showed some potential as a topical wound management material.

Keywords Poly(vinyl alcohol) · Semi-solid hydrogel · Ginger essential oil · Chitosan nanoparticles · Wound management

Introduction

Wound healing is a dynamic process that develops through three main phases; inflammatory, proliferative, and remodeling phases [1]. Since infection and dehydration of skin wound can cause chronic wound infection. Thus, the wound management has been used to protect the skin wound from infection and dehydration. To enhance the efficiency of wound healing, wound dressing must accommodate in an irregular wound and place in direct contact with the wound tissue. The properties of the ideal dressing should properly flow to fill the shape of the wound site and have sufficient adhesive properties to remove easily [2, 3]. There are various types of polymeric dressing

materials have been used in wound healing processes such as foams, films, hydrocolloids, alginates, and hydrogels. However, the hydrogel-based wound dressings were reported as the great choice materials in wound management compared to other types [4, 5].

Hydrogels are 3-dimensional networks of hydrophilic polymer chains that can retain large amounts of water. They can keep moist environment for wound and absorb extensive body fluids to assist wound healing. Moreover, the hydrogels do not adhere to the wound, which making a more comfortable dressing and reducing pain to the patient [6–8]. There are some natural polysaccharides presented in wound care as the form of hydrogels for example; alginate, chitin, and chitosan [9]. Normally, chemically crosslinked hydrogels show good elasticity and sufficient cohesive properties. However, these hydrogels are unable to flow in the wound bed. In case of physically crosslinked hydrogels, they exhibit low cohesive properties and are difficult to remove. To overcome the problems of these hydrogels, the poly(vinyl alcohol) (PVA) crosslinked with tetrahydroxyborate anions to form semi-solid hydrogels were employed. PVA is one of water-soluble polymer that can be crosslinked to form the hydrogels because it has hydroxyl group in its repeating unit to interact with other

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functional groups. The semi-solid PVA hydrogels can solve the problems such as the poor flow of hydrogels in the wound site and the difficult removal of hydrogels due to the fracture of the hydrogels [10–12]. Moreover, they are good biodegradable, good biocompatible, non-toxic, inexpensive, and non-carcinogenic. Thus, these semi-solid PVA hydrogels have the potential for use in wound management [13].

To enhance the efficiency of the semi-solid PVA hydrogels for use in wound management, a bioactive compound can be incorporated in the hydrogels. In this study, an essential oil extracted from Thai ginger plants (*Zingiber officinale* Roscoe) was used as the bioactive compound. Several studies reported some biological activities of the ginger essential oil (GEO), such as antioxidant, anti-inflammatory, and antibacterial activities, which are needed for the treatment of infected or chronic wounds [14–16]. However, to improve the physical stability, protect the interaction with other compounds, and increase the bioactivity of the essential oil, nanoencapsulation was found to be an effective method [17–19]. Nanoencapsulation of essential oil is a process whereby essential oil molecules are encapsulated inside polymeric nanoparticles. Many natural polymers can be used as encapsulating vehicles for essential oils [20]. Chitosan, due to its biocompatibility, biodegradability, non-toxicity, and inexpensiveness, is an excellent candidate as encapsulating vehicles for GEO. Chitosan nanoparticles (CNPs) exhibited good loading capacities of sensitive bioactive compounds, making them highly suitable for pharmaceutical and biomedical applications [21–24]. Amongst the various techniques used to prepare CNPs [25], ionic gelation [24] is convenient, easily processed, and free from harmful chemicals. It is based on ionic interactions between negatively-charged moieties of a polyanion (e.g., sodium tripolyphosphate (STPP) [23, 24] and positively-charged ones of chitosan.

The aim of this study was to develop the semi-solid PVA hydrogels containing GEO-loaded CNPs, to be used as a topical wound management material. The GEO-loaded CNPs were prepared using a two-step emulsion process: oil in water emulsion (O/W)/ ionic gelation technique. The GEO-loaded CNPs were characterized for their size, morphology, encapsulation efficiency (EE) and loading capacity (LC). On the other hand, the semi-solid PVA hydrogels containing GEO-loaded CNPs were characterized for their mechanical properties, adhesiveness, water content, release profiles, and indirect cytotoxicity.

Experimental

Materials

Low molecular weight chitosan (Mw = 20–30 kDa) was purchased from Bio 21 Co., Ltd. (Thailand). Ginger essential oil

(GEO, fresh pure) was purchased from Botanicesence (Indonesia). Tween 80, pharma grade was purchased from AppliChem GmbH - An ITW Company (Germany). Soybean oil (Yok Brand) was purchased from Lam Soon Public Co., Ltd. (Thailand). Span 80 was purchased from Fluka (Germany). Sodium hydroxide was purchased from LOBA Chemie Pvt. Ltd. (India). Acetic acid (glacial) 100% was purchased from Merck KGaA (Germany). Poly(vinyl alcohol) (PVA) (Mw 85,000–124,000 g/mol) was purchased from Aldrich (USA). Sodium tripolyphosphate (STPP) technical grade, 85% was purchased from Sigma-Aldrich (USA). Sodium tetraborate was purchased from Ajax Finechem (Australia). Hydrochloric acid (37%) was purchased from QREC (New Zealand). Dichloromethane (AR grade) was purchased from Macron Fine Chemicals (USA).

Preparation of ginger essential oil-loaded chitosan nanoparticles

Chitosan solution (2.0% w/v) was prepared by dissolving chitosan in an aqueous acetic solution (1% v/v) overnight at room temperature. After that, the pH of chitosan solution was adjusted to 4.4 using 2 N NaOH. Tween 80 (0.45 g) was then added to the chitosan solution (40 mL) and the solution was stirred at room temperature until the homogeneous aqueous phase solution was achieved. After that, soybean oil (10 mL) and Span 80 (0.05 g) were mixed together at 50 °C for 2 h and then cooled at room temperature. The mixture of GEO and Tween 80 in the ratio of 1:1 (v/v) was added to the mixture of soybean oil and Span 80. After that, the mixture solution was stirred to achieve a homogeneous oil phase solution. The oil phase solution (10 mL) was gradually dropped into the aqueous solution (40 mL) under homogenization at a speed of 16,000 rpm for 2 min to obtain an O/W emulsion. The ratios of chitosan: GEO [1:0 (control), 1:0.01, 1:0.03 and 1:0.05, v/v] were used in this study [22, 23].

After formation of a fine O/W emulsion, STPP solution (1.0% w/v, 40 mL) was then slowly dropped into the O/W emulsion with continuously stirring under magnetic stirrer to obtain ionic gelation. The mixture solution was then subjected to sonication for 30 min at amplitude of 80 on ice bath. The formed nanoparticles were collected by centrifugation at 10,000 rpm for 10 min at 20 °C and subsequently washed several times with Tween 80 solution (0.1% v/v) and water. The nanoparticles were lyophilized and stored in dry condition at room temperature until use.

Morphology and size determination of ginger essential oil-loaded chitosan nanoparticles

Morphological appearance of the GEO-loaded CNPs was characterized by a LEO 1450 VP Scanning Electron Microscopy (SEM; Carl Zeiss, Germany). Prior to the

investigation, the dried nanoparticles (1 mg) were dispersed in distilled water (20 mL) and then sonicated for 4 min. After that, one drop of the dispersed nanoparticles solution was placed on a stub and dried at room temperature. The dried nanoparticles were then coated with a thin layer of gold. Finally, SEM images of the GEO-loaded CNPs were obtained.

Sizes of the GEO-loaded CNPs were determined by zetasizer (Nano series, Malvern, U.K.). Each sample was dispersed in distilled water using disposal folded capillary cells with electrodes at 25 °C. Dynamic light scattering (DLS) was used to determine the average size of particles.

Determination of encapsulation efficiency and loading capacity

First, the GEO-loaded CNPs were dissolved in 5 ml of 1 M HCl in glass tubes and sonicated for 1 min. Then, 5 ml of dichloromethane was added to the solution and mixed for 30 min to extract GEO. The amount of GEO in dichloromethane was quantified using Lambda 35 UV-vis spectrophotometer (PerkinElmer, USA) at a wavelength of 235 nm and its concentration was calculated using the calibration curve. The encapsulation efficiency (EE) and the loading capacity (LC) were calculated by the following equations:

$$EE(\%) = \frac{\text{Weight of the loaded essential oil}}{\text{Weight of the initial essential oil}} \times 100 \quad (1)$$

$$LC(\%) = \frac{\text{Weight of the loaded essential oil}}{\text{Weight of sample}} \times 100 \quad (2)$$

Preparation of semi-solid poly(vinyl alcohol) hydrogels

PVA solution (4% w/v) was prepared by dissolving PVA powders in hot distilled water. Sodium tetraborate (3% w/v) solution was then poured into PVA solution and the solution was stirred until the semi-solid PVA hydrogel was obtained. After that, the semi-solid PVA hydrogel was stored in a sealed glass container at room temperature.

In the case of the semi-solid PVA hydrogel containing GEO, PVA solution (4% w/v) was mixed with GEO (0.5% w/v, based on the volume of PVA solution). After that, sodium tetraborate (3% w/v) solution was poured into the mixture solution and the mixture solution was then stirred until the semi-solid PVA hydrogel containing GEO was obtained. After that, the semi-solid PVA hydrogel containing GEO was stored in a sealed glass container at room temperature.

For the semi-solid PVA hydrogels containing different amounts of GEO-loaded CNPs, (i.e. 0.5%, 1.0%, and 1.5% w/v, based on the volume of PVA solution), the GEO-loaded CNPs were added into PVA solution.

Sodium tetraborate (3% w/v) solution was then poured into PVA solution containing GEO-loaded CNPs and the solution was stirred until the semi-solid hydrogel was obtained. The semi-solid PVA hydrogels containing GEO-loaded CNPs were stored in the sealed glass containers at room temperature. It should be noted that the semi-solid PVA hydrogels containing 0.5%, 1.0%, and 1.5% w/v of the GEO-loaded CNPs were denoted as the semi-solid PVA hydrogels containing 0.5%GEO-loaded CNPs, 1.0%GEO-loaded CNPs, and 1.5%GEO-loaded CNPs, respectively.

Texture and adhesiveness analysis

Textural properties of samples were evaluated using a Texture Analyzer (TA.XTplus) in texture profile analysis (TPA) mode. A tubular probe was compressed twice into each sample to a depth of 5 mm at a rate of 1 mm/s with a 15 s delay between compressions. The hardness and compressibility were obtained from the force-time plots produced using texture profile analysis. The hardness was defined as the force necessary to produce a given deformation and determined by the force maximum of the first positive curve of the force-time plot. The compressibility was defined as the work required deforming the product during the first compression of the probe and determined by the area under the first positive curve of the force-time plot.

Adhesiveness of samples was evaluated at room temperature using the Texture Analyzer in adhesive mode. Excised pig skin was cut along the subcutaneous–dermal interface to separate the subcutaneous fat. The epidermal side attached to the dermal side of the pig skin was assembly secured to the upper part of the instrument. 6 g of each sample was loaded in a plastic petri dish. The pig skin attached on the probe was lowered to the surface of each sample and maintained for 30 s under 0.02 N. After 30 s, the pig skin was moved upwards at 5 mm/s. The adhesiveness was defined from the force maxima of the force–time plots produced via detachment of the pig skin from the surface of sample.

Water content of PVA hydrogels containing ginger essential oil-loaded chitosan nanoparticles

Each sample (initial weight = W_h) was dried in a vacuum oven at 50 °C until a constant weight (W_d) was reached. The water content (W_c) of the hydrogels was calculated from the following equation.

$$W_c (\%) = W_h \frac{W_d}{W_h} \times 100 \quad (3)$$

Release study

The actual amounts of GEO in the hydrogels were first determined before investigating the release characteristics of GEO. Each sample was dissolved in 3 mL of 1 M HCl in glass tubes and sonicated for 1 min. Then, 3 mL of dichloromethane was added to the solution and mixed for 30 min to extract GEO. After that, 0.7 mL of sample solution was measured by a UV-vis spectrophotometer at a wavelength of 235 nm. The actual amounts of GEO in samples were then back-calculated from the obtained data against a predetermined calibration curve for GEO.

The release characteristics of GEO from each sample were investigated by the transdermal diffusion method. The release study was evaluated across a polycarbonate membrane with 8 μm pore size to allow unhindered diffusion of GEO. Cell culture inserts (Corning, USA) were filled with samples and suspended in PBS (pH 7.4) containing 0.1% *v/v* Tween 80 at 37 °C for different time points. After the specified time, 3 mL of sample was withdrawn and fresh medium was refilled. The sample solution was determined by UV-vis spectrophotometer at a wavelength of 235 nm. The obtained data were calculated to determine the released amount of GEO from the samples. The experiments were carried out in triplicate.

Indirect cytotoxicity

The indirect cytotoxicity evaluation of the samples was investigated using Normal Human Dermal Fibroblasts cell line (NHDF; 11th passage) and NCTC clone 929 cells, ATCC® CCL-1™ (11th passage). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; GIBCO, USA), supplemented by 10% Fetal Bovine Serum (FBS; GIBCO, USA) and 1% Antibiotic and Antimycotic formulation [containing Penicillin G Sodium, Streptomycin Sulfate, and Amphotericin (GIBCO, USA)] at 37 °C in a humidified atmosphere containing 5% CO₂ for 24 h. First, the samples were immersed in serum-free medium (SFM; containing DMEM and 1% Antibiotic and Antimycotic formulation) for 24 h to produce varied concentrations of extraction media (i.e., 5, 10, and 50 mg/mL) and then sterilized using 0.22 μm Minisart syringe filters (Sartorius, Germany). The NHDF or NCTC clone 929 cells were plated in wells of TCPS at 8,000 cells/well for 24 h to allow cell attachment. The cells were then starved with SFM for 24 h. After that, the medium was replaced with an extraction medium and cells were re-incubated for 24 h. The viability of the cells cultured by each of the extraction medium was determined with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay, with the viability of the cells cultured by fresh SFM was used as a control.

Statistical analysis

Data are presented as means \pm standard errors of means. Statistical analysis was carried out by one-way analysis of variance (one-way ANOVA) and Turkey's post hoc test in SPSS (SPSS, IBM, Armonk, NY, USA). The statistical significance was considered to be $p < 0.05$.

Results and discussion

Morphology of ginger essential oil-loaded chitosan nanoparticles

The morphology of GEO-loaded CNPs was examined by SEM. Figure 1 shows the SEM images of the CNPs loaded with different amounts of GEO. The results showed that the GEO-loaded CNPs mainly showed spherical shapes with aggregation. Besides, the increase of GEO amounts loaded in CNPs resulted in a smooth surface and more aggregation. Similar results for CNPs were also obtained from Feyzioglu et al. (2016) and Abreu et al. (2012) [23, 26]. In addition, the average size of the GEO-loaded CNPs at different amounts of GEO was ranging between 173 and 259 nm as shown in Table 1.

Encapsulation efficiency and loading capacity

The encapsulation efficiency (EE) and loading capacity (LC) were quantified by UV-vis spectrophotometer at a wavelength of 235 nm and calculated using Eqs. (1) and (2), respectively. %EE is the percentage of drug that successfully entrapped into the nanoparticles. From Table 1, the %EE of the GEO-loaded CNPs ranged from 21 to 45%. The %EE was found to decrease with increasing GEO content. The decrease of %EE might be corresponded due to the saturation of GEO loaded into CNPs and these results seem to be similar to the previous studies [22, 27]. The %LC of the GEO-loaded CNPs ranged from 14 to 23%. The %LC increased with increasing GEO content. From these results, the CNPs loaded with 1% *v/v* of GEO were selected to incorporate in the semi-solid PVA hydrogels because these CNPs had the highest EE.

Texture and adhesiveness analysis

The semi-solid PVA hydrogels applied to the wound area should provide a layer that is resistant to physiological stress caused by the movement of skin, as well as maintain tight contact between hydrogel and skin for a long time. Thus, the semi-solid PVA hydrogel must have the appropriate hardness and compressibility [28]. From Fig. 2(a) and (b), the hardness and compressibility of the semi-solid PVA hydrogels containing GEO-loaded CNPs were significantly lower than those of

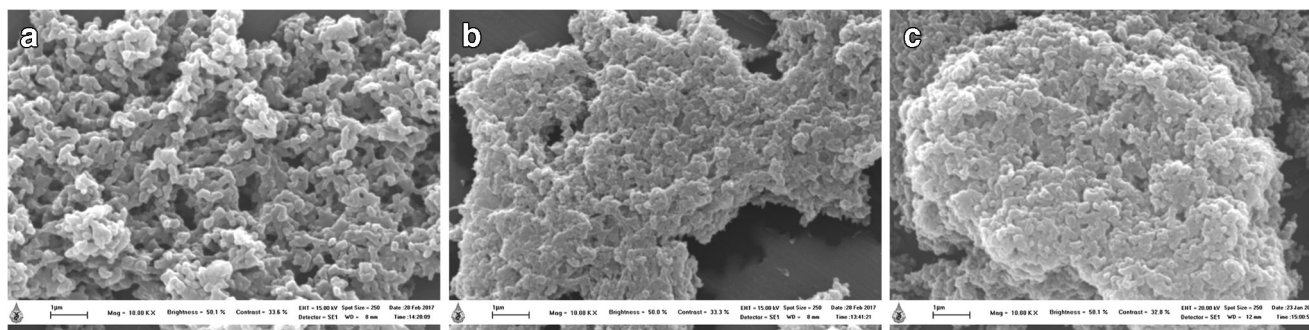


Fig. 1 SEM images of CNPs loaded with different amounts of GEO (A: 1%, B: 3%, and C: 5% v/v)

the pure semi-solid PVA hydrogels. These results might be the hydroxyl group of GEO interacted with the PVA hydrogels and increased distance between polymer chains resulting in a decrease of the electrostatic repulsion of complexation between PVA and sodium tetraborate. Thus, the viscoelasticity of the polymer chain was reduced leading to a decrease of hardness and compressibility.

To apply the semi-solid PVA hydrogels containing GEO-loaded CNPs on the wound, these hydrogels must be easily applied and removed without inducing trauma or leaving remnants [29]. Thus, the adhesiveness of the semi-solid PVA hydrogels containing different amounts of GEO-loaded CNPs was investigated and the results are shown in Fig. 2(c). The adhesiveness values of these semi-solid hydrogels were not significantly different. The adhesiveness of these semi-solid hydrogels was ranging between 3.12 and 3.72 g/cm². McCarron et al. reported that the adhesiveness of the semi-solid PVA hydrogels prepared from PVA concentration (8–19% w/w) and borax concentration (1.5–2.5% w/w) was in the range of 1.07–5.46 N/cm² [29].

Water content of PVA hydrogels containing ginger essential oil-loaded chitosan nanoparticles

The water content of the semi-solid PVA hydrogels containing different amounts of GEO-loaded CNPs is shown in Table 2. It was found that the water content in samples decreased with increasing the amount of GEO-loaded CNPs. This might be due to the high interaction between the GEO-loaded CNPs and the PVA hydrogels resulting in the decreasing interaction

of water with the PVA hydrogels. Han et al. reported that the water content of the pure PVA-borax hydrogel and the cellulose nanoparticles reinforced PVA-borax hydrogels was in the range of 95–97% [12].

Release characteristics of ginger essential oil from PVA hydrogels containing ginger essential oil-loaded chitosan nanoparticles

The actual amounts of the GEO loaded CNPs in the PVA hydrogels were determined prior to investigation the release characteristics of GEO from the hydrogels. The results showed that the actual amounts of GEO loaded in CNPs at different amounts of GEO-loaded CNPs (0.5%, 1.0%, and 1.5% w/v) were 0.01165 ± 0.00076 g, 0.01504 ± 0.00178 g and 0.01916 ± 0.00050 g.

The release characteristics of GEO from the semi-solid PVA hydrogels containing different amounts of GEO-loaded CNPs were carried out by the transdermal diffusion method over a period of 2880 min in PBS containing Tween 80 (0.1% v/v) at 37 °C. From Fig. 3, the cumulative release profiles of GEO from all hydrogels were reported as the percentage of the weight of GEO released divided by the actual amount of GEO in the samples. For the semi-solid PVA hydrogels containing GEO-loaded CNPs, the cumulative released amount of GEO from these hydrogels increased gradually with increasing dissolution time, increased and then reached a plateau value at the longest dissolution investigated time. To study the effect of incorporation of GEO-loaded CNPs in the semi-solid PVA hydrogels on the release profile of GEO, the semi-solid PVA hydrogels containing GEO were used to investigate. The results showed that the cumulative released amount of GEO from the semi-solid PVA hydrogels containing GEO more increased rapidly in the initial dissolution time, more increased afterward, and reached a plateau value at the longest dissolution time. In addition, the maximum released amounts of GEO from the semi-solid PVA hydrogels containing GEO, 0.5%GEO-loaded CNPs, 1.0%GEO-loaded CNPs, and 1.5%GEO-loaded CNPs were 91.10 ± 8.37%, 30.62 ± 8.14%, 51.05 ± 7.22%, and 72.56 ± 10.93%, respectively. From these results, the cumulative released amounts of GEO

Table 1 Particle size, encapsulation efficiency, and loading capacity of CNPs loaded with different amounts of GEO

%GEO	Particle size (nm)	EE (%)	LC (%)
0	215 ± 28	–	–
1	173 ± 26	44.60 ± 1.23	14.22 ± 0.12
3	259 ± 27	26.34 ± 1.26*	22.10 ± 0.36*
5	222 ± 52	21.03 ± 0.54*	23.26 ± 0.14*

*p < 0.05 compared with 1%GEO-loaded CNPs

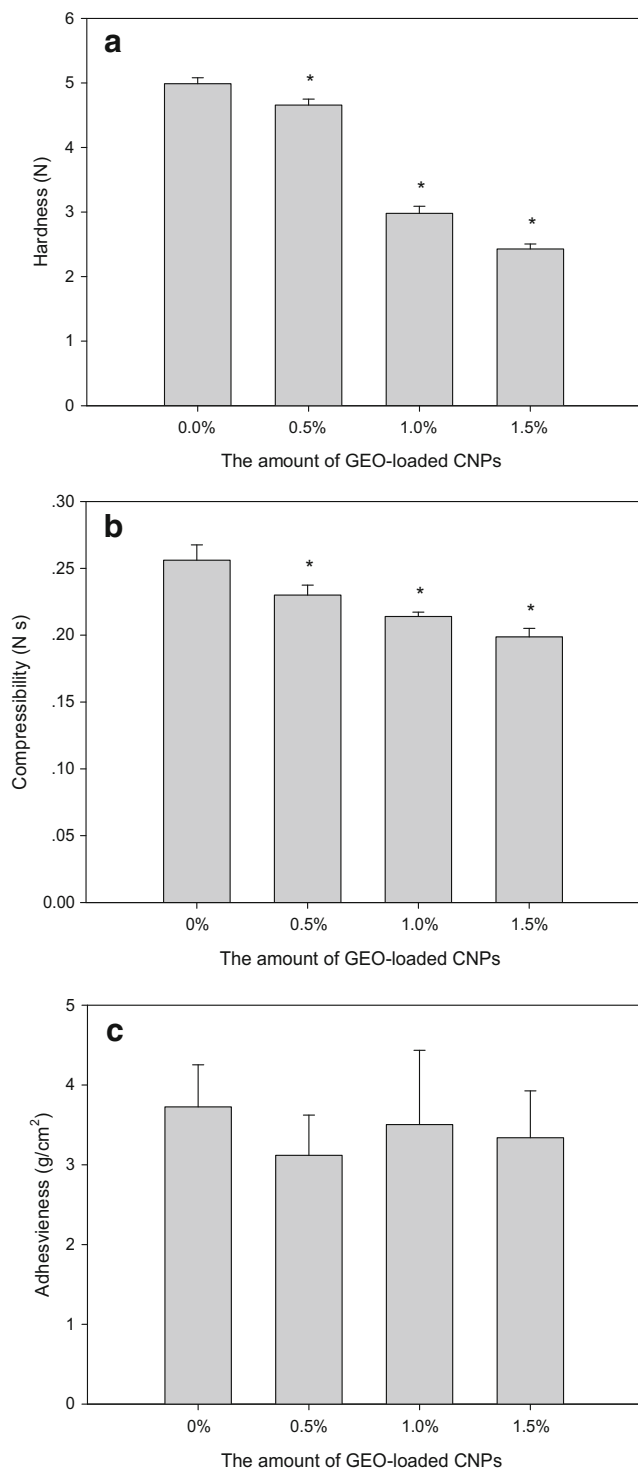


Fig. 2 **a** Hardness, **b** compressibility, and **c** adhesiveness of semi-solid PVA hydrogels containing different amounts of GEO-loaded CNPs. * $p < 0.05$ compared with pure PVA hydrogels

from the semi-solid PVA hydrogels containing GEO were greater than those from the others at all dissolution investigated time since the incorporation of GEO-loaded CNPs in the semi-solid PVA hydrogels prolonged and sustained the release of GEO [30, 31]. In addition, the cumulative released amounts

Table 2 Water content of semi-solid PVA hydrogels containing different amounts of GEO-loaded CNPs

%GEO-loaded CNPs	Water content (%)
0	96.85 ± 0.06
0.5	96.56 ± 0.02*
1.0	96.28 ± 0.02*
1.5	95.90 ± 0.05*

* $p < 0.05$ compared with pure PVA hydrogels

of GEO from the semi-solid PVA hydrogels containing 1.5%GEO-loaded CNPs were greater than those from both the semi-solid PVA hydrogels containing 0.5% and 1.0%GEO-loaded CNPs at most dissolution time because the higher amount loading of GEO resulted in a cluster of GEO-loaded CNPs on the surface of the semi-solid PVA hydrogels leading to the increased accessibility of GEO molecules to diffuse out of the semi-solid PVA hydrogels.

Indirect cytotoxicity

The indirect cytotoxicity evaluation of the samples was investigated to evaluate the potential use of these semi-solid hydrogels in wound management. From Fig. 4, the viability of NCTC clone 929 cells cultured with all concentrations of extraction media from all samples was in the range of 81–95%. While the viability of NHDF cells cultured with all concentrations of extraction media from all samples ranged between 83 and 103%. According to ISO 10993-5 in vitro cytotoxicity standard states that “reduction of cell viability by more than 30% is considered a cytotoxic effect”. From these results, the viability of both NCTC clone 929 and NHDF cells cultured with all concentrations of extraction media was higher than 70%. Thus, these semi-solid hydrogels were non-toxic to both NCTC clone 929 and NHDF cells

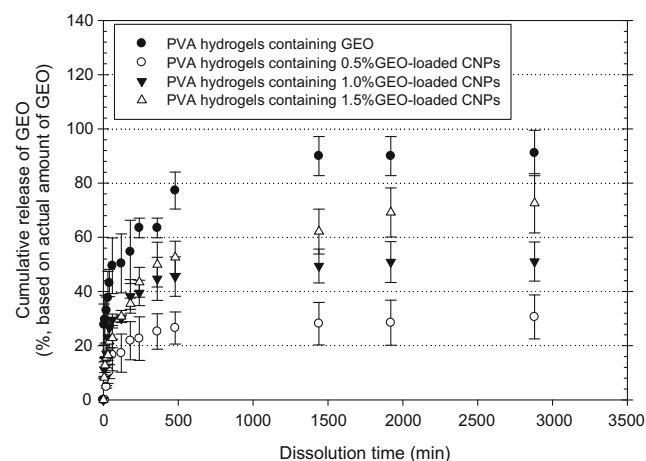


Fig. 3 Release profiles of GEO from semi-solid PVA hydrogels containing GEO and containing different amounts of GEO-loaded CNPs

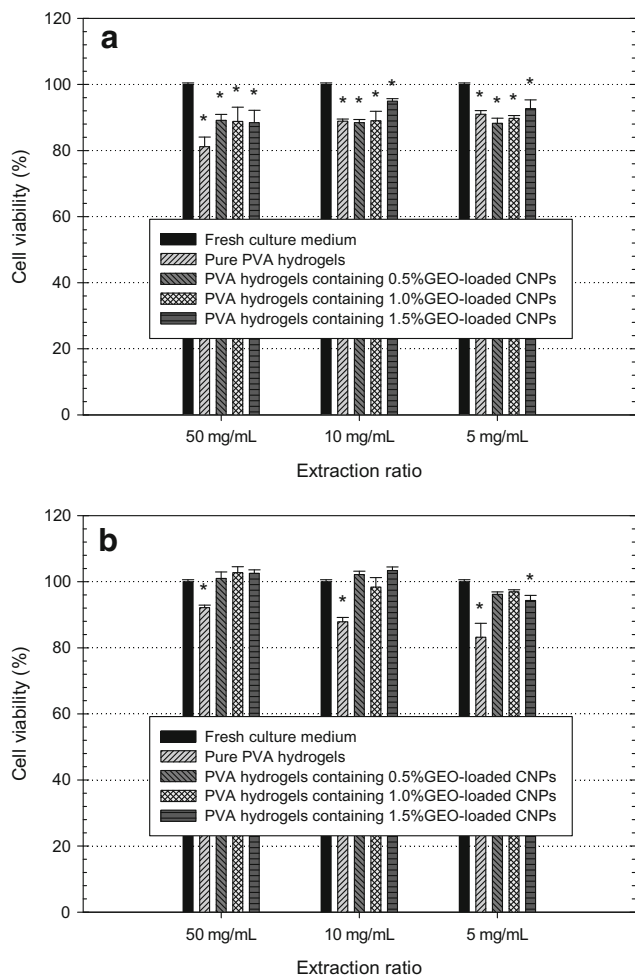


Fig. 4 Indirect cytotoxicity of pure PVA hydrogels and semi-solid PVA hydrogels containing different amounts of GEO-loaded CNPs cultured with **a** NCTC clone 929 cells and **b** NHDF cells. * $p < 0.05$ compared with fresh culture medium at any given extraction ratio

and these semi-solid hydrogels had the potential for use in wound management.

Conclusion

The GEO-loaded CNPs were successfully prepared by a two-step method: O/W emulsion and ionic gelation. The morphology of GEO-loaded CNPs was more aggregation with increasing amount of GEO in CNPs. %EE was found to decrease with increasing GEO content whereas %LC was increased with increasing GEO content. The highest %EE of CNPs loaded with 1% *v/v* of GEO was selected to incorporate within the semi-solid PVA hydrogels. The effects of the amounts of GEO-loaded CNPs in the semi-solid PVA hydrogels on hardness, compressibility, adhesiveness, water content, and release profile were studied. The hardness and compressibility of the semi-solid PVA hydrogels containing GEO-loaded CNPs decreased with increasing the amount of GEO-loaded CNPs.

The water content of the semi-solid PVA hydrogels containing GEO-loaded CNPs decreased with increasing the amount of GEO-loaded CNPs. The released amount of GEO from the semi-solid PVA hydrogels increased with increasing the amount of GEO-loaded CNPs. In addition, the incorporation of GEO-loaded CNPs in the semi-solid PVA hydrogels sustained the release rate and the released amount of GEO. Lastly, these semi-solid hydrogels were non-toxic to both NCTC clone 929 and NHDF cells.

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