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In vivo evaluation of curcumin nanoformulation loaded methoxy poly(ethylene glycol)-graft-chitosan composite film for wound healing application

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ABSTRACT

In the present study, curcumin nanoformulation loaded methoxy poly(ethylene glycol)-graft-chitosan film (curcumin-MPEG-chitosan film) was developed and its applicability in the wound healing was investigated. *In vitro* cytotoxicity test showed that the developed MPEG-chitosan film was non-cytotoxic. Antioxidant efficiency tests revealed that the antioxidant efficiency of curcumin in the film did not show any significant difference compared with that of unmodified curcumin. Furthermore, *in vivo* wound healing test showed that the rate of wound reduction was greatly elevated with the rapid re-epithelialization in curcumin-MPEG-chitosan film group. Masson's Trichrome staining and the hydroxyproline measurement in the wound tissue also suggested that application of curcumin-MPEG-chitosan film could greatly increase the collagen synthesis compared with that of MPEG-chitosan film treatment. Therefore, all these results proved the effectiveness of curcumin-MPEG-chitosan film in the application of wound healing.

1. Introduction

Generally, it is considered that the presence of reactive oxygen species (ROS) is adverse to wound healing process due to its harmful effects on cells and tissues. These ROS existed in damaged tissue will result in oxidative stress leading to lipid peroxidation, DNA breakage, and enzyme inactivation, including free radical scavenger enzymes (Martin, 1997; Singer & Clark, 1999). Previous studies have been demonstrated that application of antioxidants activities such as triterpenes, alkaloids, flavonoids, etc., could efficiently reverse the pathogenesis of many diseases leading by massive oxidants. Recently, these active principles also have been employed to promote the process of wound healing due to its excellent antioxidant activity (Chithra, Sajithlal, & Chandrakasan, 1998; Mukherjee, Verpoorte, & Suresh, 2000).

Curcumin, a yellow pigment obtained from the rhizomes of *Curcuma longa* Linn (Zingiberaceae), has been widely used for centuries in indigenous medicine for the treatment of a variety of inflammatory conditions (Ammon & Wahl, 1991). Topical application of curcumin with antioxidant activities for the treatment of wound damage has been extensively studied in past two decades (Sidhu et al., 2002). Considering the toxicity of curcumin as a high concentration in topical application, we attempted to incorporate the curcumin into MPEG-chitosan film and investigated the efficiency of curcumin-MPEG-chitosan film on the healing of cutaneous wounds in rats when administered topically. Previous studies in animal experiment have been demonstrated that the acceleration of wound healing could be gained by the increase of collagen synthesis at early phase of wound healing with chitosan treatment (Alemdaroglu et al., 2006; Jayakumar, Prabaharan, Kumar, Nair, & Tamura, 2011; Muzzarelli, 2009; Ueno et al., 1999). A number of researchers suggested that the glucosamine unite from chitosan skeleton as the activating agent of macrophage could accelerate the cytokine production from macrophage, yet promoting the wound healing (Berdal et al., 2007; Yadav & Schorey, 2006). Nishimura et al. (1984) also reported that chitosan and its derivates could stimulate the production of IL-1 by macrophage, which is strongly affects the fibroblast proliferation and collagen synthesis. Although extensive studies have been performed on wound healing with curcumin and chitosan/its derivates treatment, combined using these two active compounds has not yet been carried out. Herein, the curcumin-MPEG-chitosan film was developed and its applicability in the wound healing was evaluated.

2. Materials and methods

2.1. Materials

Chitosan, 92% degree of deacetylation (DD), with molecular weight ~200 kDa was supplied by Sigma–Aldrich (USA). Methoxy

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poly(ethylene glycol)-b-poly(e-caprolactone) copolymer (MPEG-PCL, Mn = 4000) was synthesized in our previous report (Li et al., 2010). Linoleic acid and Tween-20 were purchased from WenZhou Chemical Reagents Co. Ltd (Wenzhou, China). All the materials used in this article were analytic reagent grade. Distilled water from Milli-Q water system was used to prepare the aqueous solutions.

2.2. Preparation and characterization of curcumin nanoformulation

Due to the poor water solubility and stability of curcumin, it was first fabricated into nanoparticles, and then encapsulated into MPEG-chitosan film. Briefly, 0.2 g curcumin and 0.8 g MPEG-PCL copolymer were co-dissolved into 10 ml acetone solution, and then evaporated by a rotary evaporation at 37 °C. Finally, the resultant product was re-dissolved into 40 ml hot water solution to obtain the curcumin nanoformulation.

Particle size distribution of curcumin nanoformulation was detected by laser diffraction (Zeta plus-Zeta potential analyzer, Brookhaven Instruments Corporation, USA). Particle size was measured at 25 °C and run at least three times with independent particle batches.

The morphology of obtained curcumin nanoformulation was observed with a transmission electron microscopy (TEM) (H-6009IV, Hitachi, Japan): sample was diluted with distilled water and placed on a copper grid covered with nitrocellulose. The sample was negatively stained with phosphotungstic acid and dried at room temperature before the observation.

2.3. Preparation and characterization of curcumin-MPEG-chitosan film

In this paper, the casting/solvent evaporation method was employed to develop curcumin-MPEG-chitosan film (Li et al., 2010). Initially, 10 mg/ml of MPEG-chitosan and 5 mg/ml of curcumin nanoformulation were prepared in the distilled water, respectively. After that, 10 ml MPEG-chitosan solution and 5 ml curcumin nanoformulation solution (containing 25 mg of curcumin and 100 mg of MPEG-PCL copolymer) were mixed together and poured into a Petri dish (diameter: 6 cm), followed by drying at 50 °C for 2 days to obtain curcumin-MPEG-chitosan film. Finally, the obtained composite film was peeled from the Petri dish and divided in portions of 3 cm^2 (1.5 cm × 2 cm; containing 2.65 mg of curcumin per piece) for further application. The morphology of developed MPEG-chitosan film and curcumin-MPEG-chitosan film was further characterized by a scanning electron microscopy (JSM-5900LV, JEOL, Japan). The samples were sputtered with gold and placed at cabinet drier for 24 h before the observation.

2.4. In vitro cytotoxicity study

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test was used to determine the cytotoxicity of extraction from MPEG-chitosan films. The extraction fluids of MPEG-chitosan films were obtained by the incubation of MPEG-chitosan film (area 3 cm^{-2}) with 5 ml PBS solution for 24 h. First of all, L929 cells were cultured at DMEM medium, and then seeded onto 96-wells plates to achieve the cell concentration at 5×10^4/cells. Thereafter, a series of concentrations of MPEG-chitosan films extracts (25%, 50%, 75% and 100%, respectively) were added for 24 h's co-incubation. Finally, the absorbance at 570 nm was recorded by a Microplate reader (Bio-Rad, USA). The cell viability (%) was related to the control wells containing untreated cells with fresh cell culture medium and was calculated according to the following: cell viability (%) = absorption test/absorption control × 100%. All data are presented as the mean of six measurements (±SD).

2.5. In vitro release studies

To evaluate the release behavior of curcumin from composite film, the film samples (3 cm^2; 1.5 cm × 2 cm; containing 2.65 mg of curcumin) were first placed in an Eppendorf Centrifuge Tube (EP tube), followed by the addition of 5 ml PBS as the release medium. Thereafter, the EP tube was placed in an air shaker bath with 100 rpm/min at 37 °C for periodical study. At specific time intervals, 1 ml aliquot release medium was collected and the resultant release medium was replaced with 5 ml freshly pre-warmed PBS solution (37 °C, pH 7.4) for continuous study. Finally, the curcumin concentrations were measured by a High Performance Liquid Chromatography (HPLC, Aglilent 1200 series). HPLC analysis was performed on a reversed phase C18 column (4.6 mm × 150 mm, 5 μm, ZORBAX Eclipse XDB-C18) at room temperature. The mobile phase was composed of methanol and 0.3% acetic water solution (80:20, v/v), filtered through a 0.22 μm Millipore filter and degassed prior to use. The flow-rate was 1.0 ml/min and the eluent was detected by DAD detector at 420 nm.

2.6. Antioxidant efficiency measurement

The total antioxidant efficiency of curcumin in MPEG-chitosan film was determined by the ferric thiocyanate method as report of Tuba Ak et al. with little modification (Ak & Gül in, 2008). Briefly, curcumin released from MPEG-chitosan film in PBS (pH 7.4) solution was first quantified by HPLC method and then the inhibition of lipid peroxidation of curcumin was detected by the method as follows. Initially, the curcumin solution with concentration at 30 μg/ml was prepared in 2.5 ml phosphate buffer solution (PBS), and then mixed with 2.5 ml of linoleic acid emulsion, which was composed of 9.7 μl of linoleic acid, 11 mg of Tween-20 and 2.5 ml PBS. The resulting mixtures (5 ml) were incubated in an airtight glass bottle at 37 °C for 30 h and the absorbance at λ = 500 nm was recorded. The control group was composed of 2.5 ml linoleic acid emulsion and 2.5 ml PBS absence of curcumin. The percent inhibition of lipid peroxidation in linoleic acid emulsion was calculated by the following equation:

$$\text{Inhibition of lipid peroxidation}\,(\%) = \left(100 - \frac{A_S}{A_C}\right) \times 100$$

A_S is the absorbance of the sample in the presence of curcumin and A_C is the absorbance of the control group.

2.7. Animal experiments

Adult male SD rats weighting of 120–150 g were selected for the animal experiment. All experimental protocols and animal care complied with the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, and were approved by the Institutional Animal Care and Use Committee of Wenzhou Medical College. First of all, the dorsal hair of the rats was removed by using chloral hydrate after anaesthetization. After that, 3 cm^2 (2 cm × 1.5 cm) full thickness open excision wound was created by using a scalpel blade on the back of rats as reported in previous studies (Gopinath et al., 2004). A total of 18 animals were divided into two groups: an experimental group with treatment of curcumin-MPEG-chitosan film (film area: 3 cm^2) and a control group with treatment of MPEG-chitosan film (film area: 3 cm^2), nine rats for each group. On 3th, 7th and 14th day of post wounding, three rats

from each group were sacrificed by cervical dislocation and the wound reduction was calculated by the following formula:

$$\text{Wound reduction}(\%) = \frac{\text{Wound area day 0} - \text{Wound area day } t}{\text{Wound area day 0}} \times 100$$

where wound area day 0 was the original wound area after the surgery and wound area day t was the wound area on t days of post wounding.

2.8. Histological observation

On 3th, 7th and 14th day of post wounding, the rats from both MPEG-chitosan film group and curcumin-MPEG-chitosan film group were sacrificed, respectively. Tissues from wound site were removed by a surgical scissors and subsequently fixed into 10% formalin, dehydrated through graded alcohol series, cleared in xylene and embedded in paraffin wax. Serial sections of 3 μm were cut, and stained with hematoxylin and eosin (H&E) for general observation of microstructure changes of wound tissue. Masson's Trichrome method for staining collagen was performed on paraffin sections, as described by previously (Sidhu et al., 1999). Collagen is stained blue with Masson's Trichrome stain. Furthermore, hydroxyproline assay was performed as a marker of collagen synthesis in wound tissues using the method previously described with little modification (Blumenkrantz & Asboe-Hansen, 1975). Wound tissues were harvested at specific time points (3th, 7th and 14th day of post wounding) and dried at 110 °C overnight, then hydrolyzed with 2 M NaOH for 30 min at 120 °C, which was followed by the determination of hydroxyproline by modification of the Neumann and Logan's reaction using chloramine T and Ehrlich's reagent, using a hydroxyproline standard curve and measuring at 550 nm. Values were expressed as micrograms of hydroxyproline per milligram of dry weight tissue.

2.9. Statistical analysis

Data was analyzed using the software program Origin Pro 7.5. Statistical comparison between curcumin-MPEG-chitosan film and MPEG-chitosan film was determined by using one-way ANOVA using SPSS software ($p < 0.05$).

3. Results and discussion

3.1. Preparation and characterization of curcumin nanoformulation

The single-step nano-precipitation method was employed to fabricate the curcumin nanoformulation using the MPEG-PCL copolymer as the carrier. As presented in Fig. 1A, we could clearly observe that the developed curcumin nanoformulation with concentration at 5 mg/ml (Fig. 1A-b) was a very fine dispersion and appeared to be soluble, unlike that of curcumin (Fig. 1A-a), which was completely insoluble in water solution due to the very low solubility (20 μg/ml), with undissolved flakes clearly visible in the suspension. In this formulation, except the curcumin and MPEG-PCL copolymer, there was absence of any stabilizers or surfactants in formulation, which might be advantage for the further *in vivo* application. Meanwhile, the morphology of resulting curcumin nanoformulation was observed by a transmission electron microscopy (TEM), as shown in Fig. 1B. According to the Fig. 1B, it revealed that the developed curcumin nanoformulation was spherical and monodisperse with a mean diameter of about 40 nm. Interestingly, these nanoparticles did not show any aggregation or adhesion among these particles in spite of absence of any stabilizers or surfactants in the formulation. However, the particle size of curcumin nanoformulation measured by dynamic light scattering was about 50 nm with polydispersity index (PDI) about 0.102, as shown in Fig. 1C. The discrepancy in size of the nanoparticles when comparing the diameter obtained by TEM observation with that obtained by particle-size analyzer was attributed to the fact that what was measured by the dynamic light scattering method was the hydrodynamic diameter of nanoparticles in aqueous solution, whereas the TEM observation revealed the morphology size of nanoparticles in solid state.

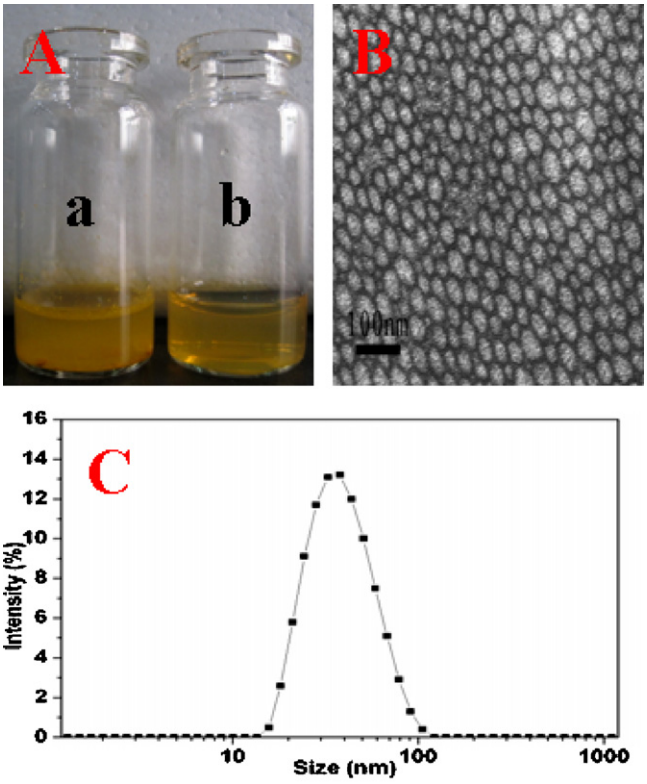

Fig. 1. Preparation and characterization of curcumin nanoformulation: (A) appearance of curcumin water dispersion (a) and curcumin nanoformulation (b); (B) TEM image of curcumin nanoformulation; (C) size distribution of curcumin nanoformulation.

3.2. Preparation and characterization of curcumin-MPEG-chitosan film

In this paper, curcumin-MPEG-chitosan films were successfully developed by the casting/solvent evaporation method. As depicted in Fig. 2, it clearly observed that the developed curcumin-MPEG-chitosan film (Fig. 2B) appeared saffron yellow color without any visible curcumin separation, indicating that the curcumin nanoformulation was uniformly dispersed into composite films, while the MPEG-chitosan film (Fig. 2A) showed white color due to the presence of MPEG-PCL copolymer in the composite film. More specifically, the developed composite films were characterized by a SEM observation, and the results were presented in Fig. 2C and D. According to Fig. 2, we could clearly observe that the curcumin-MPEG-chitosan film appeared to roughness on the surface absence of any pore structure (Fig. 2D-1), which was no obvious discrepancy with that of MPEG-chitosan film (Fig. 2C-1), indicating that incorporation of curcumin nanoformulation to the MPEG-chitosan film did not influence the surface morphology of composite film. For the cross-section of composite films, it clearly observed that the curcumin-MPEG-chitosan film was about 30 μm (Fig. 2D-2) with no obvious separation of curcumin might be suitable for the wound healing application. Meanwhile, it also clearly showed that

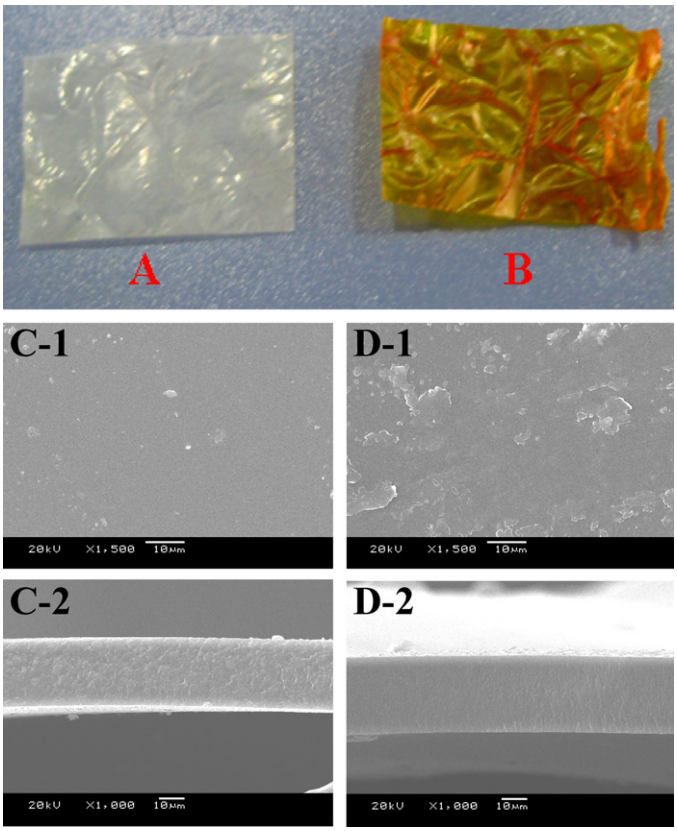

Fig. 2. Optical photograph of composite film (A: MPEG-chitosan film; B: curcumin-MPEG-chitosan film); SEM observation of composite films: (C-1) surface of MPEG-chitosan film; (C-2) cross-section of MPEG-chitosan film; (D-1) surface of curcumin-MPEG-chitosan film; (D-2) cross-section of curcumin-MPEG-chitosan film.

the developed MPEG-chitosan film and curcumin-MPEG-chitosan film were dense structure absence of any pore structure, which was in accordance with our previous study (Li et al., 2010).

3.3. *In vitro* cytotoxicity study

Previous studies have been demonstrated that the MPEG-chitosan and MPEG-PCL copolymer were non-cytotoxicity against ROS cells after 24 h incubation (Li et al., 2010). However, the cytotoxicity of MPEG-chitosan film should be evaluated before its further *in vivo* application. In this paper, L929 cells were employed to evaluate the intrinsic cytotoxicity of MPEG-chitosan films by measuring the cell viability after 24 h's co-incubation with its extract fluids. As presented in Fig. 3, it showed that all samples (25%, 50%, 75% and 100% extraction) were non obvious cytotoxic to the L929 cells after 24 h's incubation, suggesting that the developed MPEG-chitosan films was non-cytotoxic suitable for the further application.

3.4. *In vitro* release studies

In this paper, *in vitro* release behavior of curcumin from MPEG-chitosan film was performed in PBS solution at 37 °C and the result was presented in Fig. 4. According to Fig. 4, we could find that an initial release of 8.4% of total drug encapsulation was observed in 1 day, followed by a sustained release for a period of 5 days to achieve the plateau with about 40% of total drug encapsulation. The initial quick release of curcumin from composite film might be attributed to that the curcumin molecules dispersing close to the polymer film surface were those adsorbed at or loosely bound near the surface, which diffused in the initial incubation time (Cui et al., 2006).

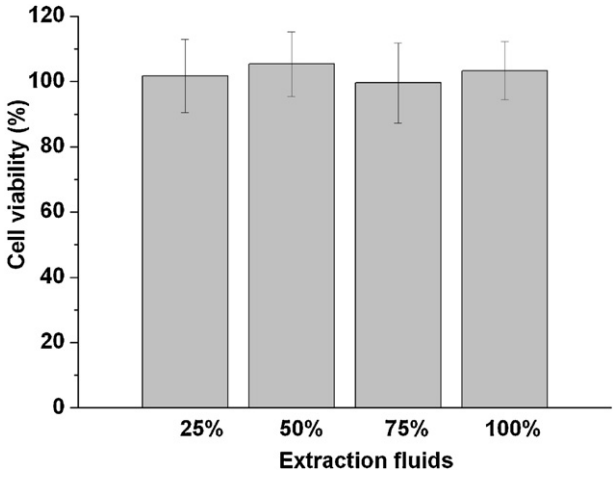

Fig. 3. *In vitro* cytotoxicity of extractions from MPEG-chitosan films against L929 cells after 24 h incubation.

However, the majority of curcumin (about 60% of total drug encapsulation) still remained inside the composite film after one week implying that there was presence of possible interaction between curcumin nanoformulation and MPEG-chitosan film. Our previous study has been demonstrated that there was presence possible interaction (hydrogen bond) between MPEG-chitosan and MPEG-PCL co-polymer (Li et al., 2010). In the case of MPEG-chitosan and MPEG-PCL co-polymer, previous studies have been demonstrated that both of two polymers could be slowly degraded or adsorbed as topical administration with the time evolution. Therefore, the resident curcumin could be released slowly with the degradation of matrix polymer occurred.

3.5. *Antioxidant efficiency measurement*

Previous studies have been demonstrated that the reactive oxygen species (ROS) in the wound tissue could lead to oxidative modification cellular membrane or intracellular molecules resulting in the tissue damage (Maheshwari, Singh, Gaddipati, & Srimal, 2006). Therefore, the administration of antioxidant agents such as curcumin topically was essential for the promotion of wound healing. Although numerous studies on the antioxidant efficiency of curcumin have been performed, the curcumin incorporation in the composite films has not been carried out. In this article, the antioxidant efficiency of curcumin in composite film was

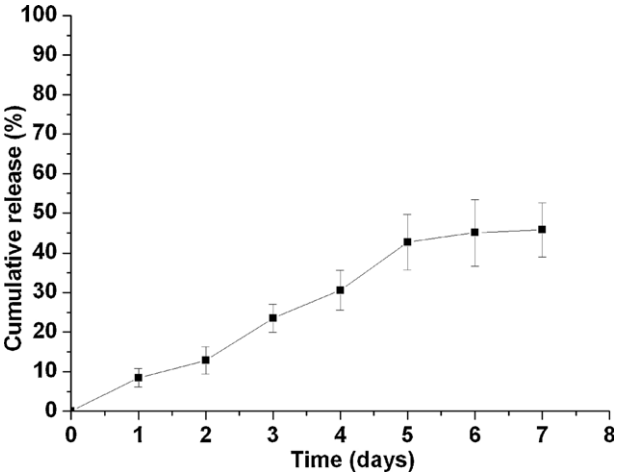

Fig. 4. *In vitro* release profile of curcumin from MPEG-chitosan films in PBS solution (pH 7.4) at 37 °C.

Table 1
Total antioxidant activity of unmodified curcumin and curcumin in MPEG-chitosan film with concentration at 30 μg/ml.

Sample	Inhibition of lipid peroxidation (%)
Unmodified curcumin	99.23 ± 0.45
Curcumin in MPEG-chitosan film	97.49 ± 0.61

measured by ferric thiocyanate method as reported previously. As shown in Table 1, curcumin released from MPEG-chitosan film (30 μg/ml) exhibited excellent antioxidant efficiency with about 97% of inhibition of lipid peroxidation similar to that of unmodified curcumin (about 99%), indicating that the encapsulation of curcumin nanoformulation into MPEG-chitosan film did not influence its intrinsic antioxidant efficiency. This result suggested that the developed curcumin-MPEG-chitosan film with excellent antioxidant efficiency might have potential application in the wound healing.

3.6. Animal experiments

Chitosan as a biologically active dressing has been widely applied in the wound management and its related product has been marketed (Tegaderm™ Film) (Muzzarelli, 1993; Muzzarelli, Mattioli-Belmonte, Pugnaloni, & Biagini, 1999; Muzzarelli et al., 2007). Recently, numerous studies showed that topical administration of curcumin could greatly promote the wound healing. Herein, we combined using MPEG-chitosan and curcumin to develop a novel curcumin-MPEG-chitosan film and its potential application in the wound healing was investigated. To understand mechanism of wound healing, several models including dead space chamber, punch models, linear incision model, and full-thickness punch biopsy models have been employed to evaluate the rate of wound healing (Sidhu et al., 1998, 1999). Each model has several advantages and disadvantages over the other. Here, we adopted full-thickness punch wounds to evaluate the efficiency of curcumin-MPEG-chitosan film on the wound healing. Fig. 5A depicts the wound healing rate of rats from both MPEG-chitosan film group and curcumin-MPEG-chitosan film group, respectively. Due to the great water adsorption and excellent adhesive property of composite film, the bleeding of wound was ceased immediately (data not shown) and formed an adhesive film in situ after topical application of composite film. With the time proceeding, the MPEG-chitosan film as well as the curcumin-MPEG-chitosan films was found to be rapidly degraded/absorbed after 1 week of application as observed in optical photography (Fig. 5A), which is in accordance with the results of its biodegradability as identified by *in vitro* degradation test. From Fig. 5B, we could find that the wound healing rate of rats from curcumin-MPEG-chitosan film group showed significant difference compared with that from MPEG-chitosan film group on 3th, 7th and 14th day of post wounding ($n = 3$; $p < 0.05$). Healing of rats from both MPEG-chitosan film and curcumin-MPEG-chitosan films groups were found to be immediately after the wounding (Fig. 5B). More specifically, the wound reduction of rats from MPEG-chitosan films was about 62% on 3th day of post wounding, whereas the wound reduction of rats from curcumin-MPEG-chitosan films was about 80% on 3th day of post wounding. The initial phase of wound healing might be induced by the adsorption of various protein molecules from the wound surface into the MPEG-chitosan film resulting in the healing of wound tissue (Obara et al., 2003). After that, due to the sustained release of curcumin from composite films, the wound healing of rats from curcumin-MPEG-chitosan films group was greatly enhanced with approximately 90% wound reduction after 2 week of treatment (Gopinath et al., 2004; Grinnell, 1994; Sidhu et al., 1998), whereas only about 60% wound reduction was observed in the rats of

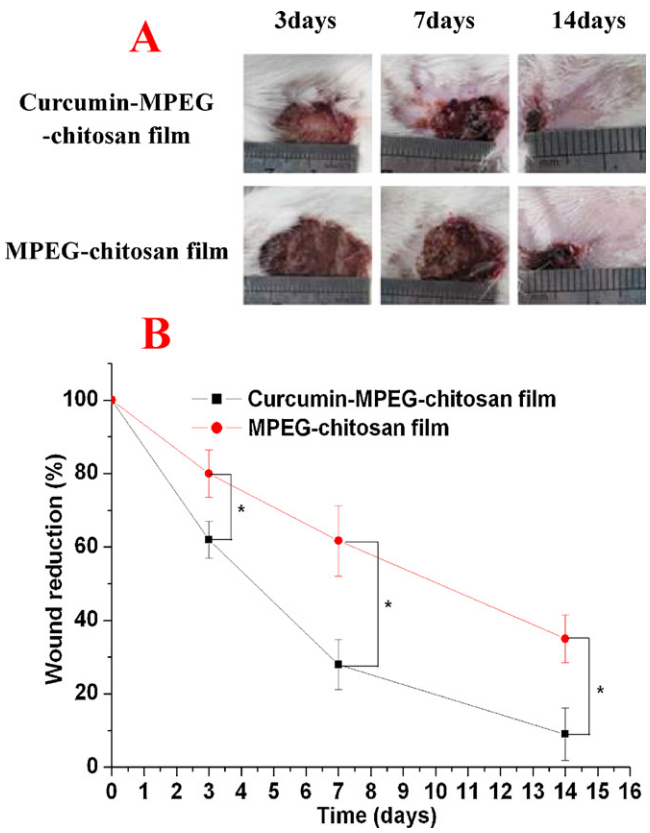

Fig. 5. (A) The photographs of the wound at 3th, 7th and 14th day are representative of three rats in each group. (B) Wound reduction of MPEG-chitosan film and curcumin-MPEG-chitosan film treated SD rats. The data represent the mean ± SD of three rats. The statistical significance of wound reduction was evaluated on 3th, 7th and 14th of post-wounding. *One-way ANOVA analysis, $p < 0.05$, $n = 3$.

MPEG-chitosan film treatment, suggesting that application of curcumin could greatly promote the rate of wound healing.

3.7. Histological study

Fig. 6 depicts the histopathological changes of wound tissue from both MPEG-chitosan film group and curcumin-MPEG-chitosan film group as a function with time. On 3th day of post wounding, film residual, nonviable necrotic areas as well as the inflammatory cells were found in the wound area of both two groups, while curcumin-MPEG-chitosan film treated wounds showed an increase in the infiltration of cells and granulation generation. Seven days later, a migration of the epithelium over the dermis was observed along with the granulation tissue formation both in the wound of MPEG-chitosan film group and curcumin-MPEG-chitosan films group. However, the complete re-epithelialization of wound was observed in rats of curcumin-MPEG-chitosan films group, indicating that the combined using curcumin nanoformulation could significantly accelerate the re-epithelialization of wound. On 14th day of post wounding, we could find that the complete epithelialization of the wound was observed in rats of curcumin-MPEG-chitosan films group, yet defective epithelialization of the wound was seen in rats of MPEG-chitosan film group, which is in accordance with the our naked eye observation (Fig. 6).

The collagen deposition in the wounds was examined by Masson's Trichrome staining and hydroxyproline assay as shown in Fig. 7. From Fig. 7A, it revealed that the wound of curcumin-MPEG-chitosan film group showed a greater collagen content no matter at 3th day or at 7th day and 14th day as compared with

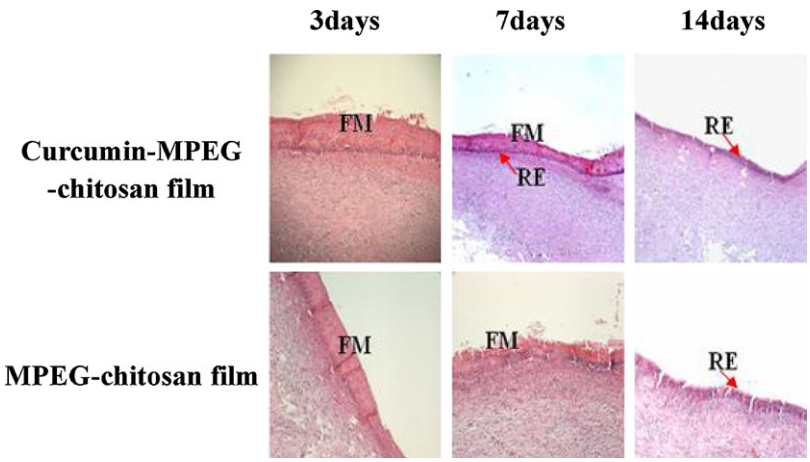

Fig. 6. HE stained section of the wound with MPEG-chitosan film and curcumin -MPEG-chitosan film treatment on 3th, 7th and 14th day of post wounding (200×). FM: film; RE: re-epithelialization.

that of MPEG-chitosan film group. Moreover, the wound of rats in curcumin-MPEG-chitosan film group had a compact and well aligned collagen, whereas collagen was loose and irregular arrangement in rats of MPEG-chitosan film group. As well know to us, the collagen generation in wound area is very important for the remodeling of tissue as well as tissue tensile strength. Meanwhile, the hydroxyproline content in wound tissue from each group was determined as a function with time, as presented in Fig. 7B. It clearly observed that the actual collagen deposition as measured by hydroxyproline content was significantly higher in curcumin-MPEG-chitosan group as compared with that of MPEG-chitosan groups on 7th and 14th day of post wounding, which was in accordance with result of Masson's staining. Therefore, all these results concluded that topical application of curcumin-MPEG-chitosan film could significantly accelerate the wound reduction and healing.

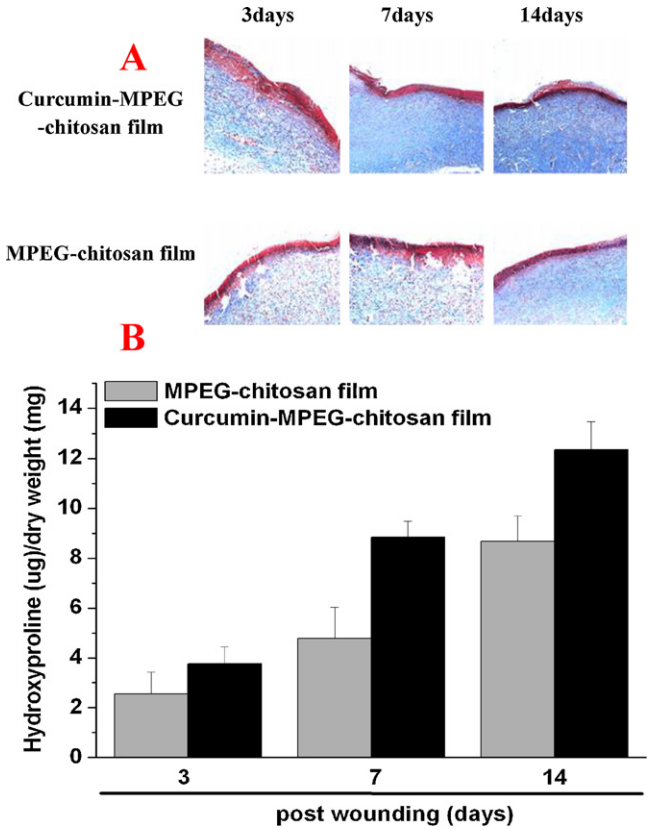

Fig. 7. (A) Masson's Trichrome staining of the wound with MPEG-chitosan film and curcumin-MPEG-chitosan film treatment on 3th, 7th and 14th day of post wounding (200×). (B) Total amount of hydroxyproline in wound tissue of MPEG-chitosan film and curcumin-MPEG-chitosan film on 3th, 7th and 14th day of post wounding. Data represent mean ± SD of three rats.

4. Conclusion

In this article, a novel curcumin nanoformulation loaded MPEG-chitosan film was developed and the potential application of wound healing was evaluated. Because of the poor water solubility of curcumin, curcumin was first fabricated into nanoformulation based on MPEG-PCL co-polymer resulting in the completely soluble in water solution with a high concentration at 5 mg/ml. Incorporation of curcumin nanoformulation into MPEG-chitosan film did not influence the its intrinsic antioxidant efficiency as compared with that of unmodified curcumin. *In vitro* release behavior of curcumin from composite film suggested that curcumin was sustained release from composite film with about 44.5% of total drug encapsulation released after 1 week study. In full-thickness punch wounds model of SD rats, topically administrated curcumin-MPEG-chitosan film is effective in faster wound reduction and healing compared with that treated with MPEG-chitosan film. Furthermore, the period of re-epithelialization was shorter in the curcumin-MPEG-chitosan film treated wounds. The result of Masson's Trichrome staining indicated that the curcumin-MPEG-chitosan film wound had a compact and well aligned collagen, whereas collagen was loose and irregular arrangement in MPEG-chitosan film wound. All these results suggested that the developed curcumin-MPEG-chitosan film had great potential application in the wound healing.

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