Injectable nanocurcumin-dispersed gelatin–pluronic nanocomposite hydrogel platform for burn wound treatment

LE HANG DANG^{1,2,†}, NGOC TRINH HUYNH^{3,4,†}, NGOC OANH PHAM^{1,3},
CONG TRUC NGUYEN³, MINH THANH VU⁵, VAN THOAI DINH^{2,6}, VAN THU LE^{2,3}
and NGOC QUYEN TRAN^{2,3,6,*}

¹School of Biotechnology, International University, National Universities in HCMC, 70000 Ho Chi Minh City, Viet Nam

²Graduate University of Science and Technology, Ho Chi Minh City 700000, Viet Nam

³Institute of Applied Materials Science, Viet Nam Academy of Science and Technology, Ho Chi Minh City 700000, Viet Nam

⁴Faculty of Chemistry, Tra Vinh University, Tra Vinh Province 940000, Viet Nam

⁵Institute of Chemistry and Materials, 17 Hoang Sam 100000, Cau Giay, Ha Noi, Viet Nam

⁶NTT Hi-Tech Institute, Nguyen Tat Thanh University, District 4, Ho Chi Minh City 700000, Viet Nam

*Author for correspondence (tnquyen@iams.vast.vn)

† First two authors contributed equally to this study.

MS received 13 May 2018; accepted 16 August 2018; published online 6 March 2019

Abstract. To utilize the potent pharmaceutical properties of curcumin (Cur) and gelatin-based materials in tissue regeneration, we fabricated a thermosensitive nanocomposite hydrogel based on pluronic-grafted gelatin (PG) and nanocurcumin (nCur) to enhance burn healing. In this method, the amphiphilic PG played a role as a surfactant to prepare and protect nano-sized Cur particles, which could overcome the poor dissolution of the phytochemical. The synthesized PG was identified by ¹H nuclear magnetic resonance. Depending on the amount of Cur, size distribution of the dispersed nCur ranged from 1.5 ± 0.5 to 16 ± 3.2 nm as observed using transmission electron microscopy and dynamic light scattering. The nCur-dispersed PG solution formed nCur–PG nanocomposite hydrogel on warming up to 35°C. Release profile indicated sustainable release of Cur from the injectable platform. Fibroblast cells were well proliferated on the nanocomposite hydrogel. The nCur–PG enhanced the healing process of second-degree burn wound. These results showed potential applications of the biomaterial in tissue regeneration.

Keywords. Nanocurcumin; gelatin; pluronic F127; nanocomposite hydrogel; wound healing.

1. Introduction

Nowadays, wound and burn healing fields are gaining significant attention in multidisciplinary studies expanding from traditional herb to advanced biomaterials or their formulations [1,2]. Several kinds of phytochemicals have recently received much attention in the field due to their broad-spectrum bioactivities [1,2]. Among them, curcumin (Cur), an active substance in turmeric, exhibits multiple pharmacological properties such as anti-inflammatory, anti-infectious, anti-tumoural and anti-oxidation activities as well as positive effects in wound or burn healing [3–5]. In wound healing applications, several reports have indicated that Cur treatment reduces healing time in puncture wound models by improving the restoration of structural epidermis and enhancing deposition of collagen as well as vascular density in wound sites leading to increased healing effects [4,6]. However, free Cur is highly hydrophobic and is poorly absorbed leading to low bioavailability within the body that partially limits its biomedical applications [1,7]. To improve its dispersion

and bioavailability, various new Cur-dispersed formulations in the polymeric or hydrogel platforms and its conjugated derivatives have been developed, for example, Cur–chitosan–alginate blend [8], Cur-loaded poly(ϵ -caprolactone)-poly(ethylene glycol)-poly(ϵ -caprolactone) hydrogel [9], Cur-conjugated hyaluronic acid [10], etc. All these formulations showed its healing ability and its potential in biomedical applications. However, it is difficult to obtain homogeneous materials due to low Cur dispersion. Regarding this, there is some evidence indicating that Cur-encapsulated platforms for topical applications exhibit a higher effect on wound healing than its oral administration [11,12].

Interestingly, several reports have indicated that using nano-sized Cur improved Cur bioavailability and dispersion. Following this approach, many methods were used to fabricate Cur nanoparticles, such as low flow injection, evaporation, precipitation or nanosuspension [13]. Various surfactants were exploited to fabricate nanocurcumin (nCur) [14–16]. The surfactant interactions may cause dramatic changes in the solubilizing capacity of hydrophobic drugs, rheological

properties of polymer aqueous dispersions and in drug diffusion and penetration through the skin and mucous. Consequently, incorporation of the polymeric surfactant opens a wide range of possibilities for developing drug-delivery systems [16,17]. Up to now, pluronic or poloxamer has been one of the best surfactants. Pluronic consists of hydrophilic poly(ethylene oxide) (PEO) and hydrophobic poly(propylene oxide) (PPO) blocks arranged in an A–B–A tri-block structure (PEO–PPO–PEO) that is well-known for its fast thermally reversible property and being an Food and Drug Administration (FDA)-approved copolymer [18]. Because of having both hydrophobic and hydrophilic domains, pluronic displays surfactant properties in interactions with hydrophobic drugs and cellular membranes that play a vital role in drug-delivery platforms. Notwithstanding the evidence, some drawbacks of Pluronic F127-based hydrogels include their weak mechanical strength, rapid erosion (dissolution of the surface), non-biodegradability at high concentrations and limited bio-compatibility [19]. Therefore, a recent approach has utilized the pluronic-grafted copolymers to overcome the mentioned drawbacks [20,21].

In this study, we prepared a thermo-responsive pluronic-grafted gelatin (PG) copolymer as a dispersant platform for fabricating nCur under assisted sonication. The colloidal PG copolymer solution could form an injectable nanocomposite hydrogel at body temperature that may be useful in tissue regeneration due to beneficial properties of Cur and gelatin-based materials. Gelatin has gained much attention in tissue engineering because of its high biocompatibility and biodegradability as it contains Arg–Gly–Asp (RGD) sequences that promote cell adhesion and migration [22,23]. These factors could promote the wound-healing process. Gelatin-based hydrogel indicated a higher wound contraction and re-epithelialization [24]. Therefore, a combination of Cur nanoparticles and the injectable gelatin-based hydrogels could offer multifunctional biomaterials for second-degree burn treatment.

2. Materials and methods

2.1 Materials

Porcine gelatin (bloom 300), Pluronic F127 and Cur were purchased from Sigma Aldrich (St. Louis, USA). Mono *p*-nitrophenyl chloroformate-activated pluronic (NPC-P-OH) was prepared in our previous study [25]. Diethyl ether was obtained from Scharlau's Chemicals (Spain), tetrahydrofuran was purchased from Merck (Germany) and dialysis membranes (MWCO 14 kDa and MWCO 3.5 kDa cut-off) were supplied from Spectrum Labs (USA).

2.2 Synthesis of PG copolymers

In this study, four GP copolymers were prepared at different ratios of gelatin and pluronic 1:05, 1:10, 1:15 and 1:20

wt/wt. Briefly, in a round flask, gelatin (1 g) was dissolved in deionized (DI) water. An aqueous NPC-P-OH (15 g) solution was added drop-wise into the flask at 20°C under stirring overnight. Then, the mixture was dialysed against distilled water for 3 days using a cellulose membrane (MWCO 14 kDa) and lyophilized to obtain the powder as a thermo-sensitive copolymer platform for further study as seen in figure 1. Grafting yield of samples obtained around 75–80% wt/wt. The copolymer was characterized using ¹H NMR spectrum and Fourier-transform infrared (FT-IR) spectrum.

2.3 Sol–gel transition behaviour

Aqueous copolymer solutions of 0.5 ml were prepared from varying the PG samples (ratio of G:P = 1:10, 1:15 and 1:20 wt/wt) at 20°C. The designated range temperature was set up at 4, 25, 30, 37, 40 and 50°C to determine the sol–gel transition behaviour of nanocomposite hydrogel using the test tube inversion method which could observe the 'flow as the liquid solution' or 'no flow as the gel formation'. Sol–gel phase diagram was built using the recorded data.

2.4 Biodegradation test

To characterize the degradation, 1 ml of 20 w/v% copolymer samples were dissolved in phosphate-buffered saline (PBS) at 20°C and poured into test tubes. The samples were equilibrated in a water bath at 37°C and then 5 ml was added into the gel-containing test tubes. At pre-determined time intervals, the samples were removed from the buffer, dried and weighed and a fresh PBS solution was added into the tubes with the same volume. Degradation rate was recorded via a mass difference between each time point, computed by using equation (1), in which W_i is initial dry weight and W_t is dry weight at each time point. Data point was performed three times and expressed as mean ± SE:

$$\text{Weight loss (\%)} = \frac{W_i - W_t}{W_i} \times 100. \quad (1)$$

Fabrication of nCur-dispersed PG copolymer and its nCur-PG form 2.5 mg Cur which was dissolved in 5 ml absolute ethanol under sonication. The suspension was added drop-wise to the PG copolymer solution (500 mg PG in 2.5 ml DI water and 5 ml ethanol). Then, ethanol solvent was evaporated by the rotary evaporator to obtain a homogeneous nCur-loaded PG paste form and cold DI water was added to obtain thermosensitive nCur-dispersed PG copolymer solution that could transfer into nCur–PG on warming. Morphology of nCur was observed using transmission electron microscopy (TEM) (JEM-1400 JEOL) at 25°C. Spectral analysis was observed by using UV–Vis spectroscopy (Agilent 8453 UV–Vis Spectrophotometer) at 420 nm wavelength. Particle size distribution was determined using dynamic light scattering (DLS).

Figure 1. Synthetic scheme of PG copolymers.

2.5 Release study

In this study, a diffusion method with a dialysis membrane was used to investigate the *in vitro* release of Cur from the nCur-loaded composite hydrogel that was prepared from 1 ml of copolymer (20 w/v%) containing 2.5 mg nCur. The dialysis bag (MWCO 3.5 kDa) containing 2 ml sample was immersed in 10 ml PBS at $37 \pm 0.5^\circ\text{C}$ in a water bath. At selected time intervals, 1 ml of sample was collected and replaced by an equal volume of fresh medium. The Cur content was quantified by using an Agilent 8453 UV-Vis Spectrophotometer. The release experiments were performed in triplicate with 95% confidence interval. The cumulative release of drug was obtained from the below equation [26]:

$$Q = C_n V_t + V_s \sum C_{n-1}, \quad (2)$$

where C_n represents the concentration of drug in sample, C_{n-1} is release concentration at t , V_t the incubated medium and V_s the volume of replaced medium.

2.6 Biocompatibility test

According to our screening experiments on behaviour of fibroblast with different Cur concentrations as well as application of the nCur-loaded PG in tissue regeneration, nCur was loaded in the PG hydrogel at low concentration. Two kinds of freeze-dried PG hydrogel and nanocomposite hydrogel containing 0.5 wt/wt% of nCur were soaked in 1 ml (15%) of

Dulbecco's Modified Eagle Medium and incubated at 37°C for 24 h. Then, $\sim 3 \times 10^4$ fibroblast cells were seeded per well of a 24-well plate with overnight incubation before being incubated with these prepared materials similar to the previous procedure for 48 h. Treated cells were fixed with cold 50% (w/v) trichloroacetic acid solution for 2 h, washed and stained with 0.2% (w/v) sulphorhodamine B (SRB) for 20 min. After five washes with 1% acetic acid, protein-bound dye was solubilized in 10 mM Tris base solution and the absorption at 620 nm on a microplate reader was recorded. Based on the standard curve which was obtained by various amounts of fibroblasts, we calculated the amount of fibroblast cells on the samples.

2.7 Wound-healing testing on animal model

2.7a Animals: Healthy adult male *Mus musculus* var. Albino mice (33–42 g, $n = 6$) were procured from the Pasteur Institute in Ho Chi Minh city, Viet Nam. Mice were maintained in standard laboratory conditions with *ad libitum* access to feed and water, light–dark cycles and adequate ventilation.

2.7b Wound creation: The experiment was conducted at Laboratory of Department of Physiology and Animal Biotechnology under permission of the Animal Care and Use Committee of the University of Science, Vietnam National University at Ho Chi Minh City (registration no. 10/16-010-00), Viet Nam. The mice were anesthetized by intraperitoneal ketamine (100 mg ml^{-1}) and xylazine (20 mg ml^{-1}) injection

Figure 2. (a) FT-IR spectra and (b) ¹H NMR spectrum of PG copolymer compared with the original material.

with a dosage of 0.2 ml×3 100 g×3 body weight. The dorsal skin of the animals was shaved and cleaned with ethanol (70%) and polyvinylpyrrolidone iodine (1%). A second-degree burn was created by a cylindrical stainless steel rod of 1 cm diameter which is heated in boiling water at 100°C. The rod is maintained in contact with the animal skin on the dorsal proximal region for 5 s. Thereafter, medication was initiated for these four groups (non-treatment, dressing PG, nCur-PG copolymer (20 w/v%) containing 2.5 mg nCur and commercial product/Biafine). Dressings were performed for every 2 days and completed on day 14. Each mouse contained two wounds with random treatments. Wound was examined on days 0, 2, 6, 8, 12 and 14. Wound size was measured using a Caliper (0–200 mm Mitutoyo 530-114). The area of wound contraction was calculated following the equation [27]:

$$\text{Area of wound} = \frac{\pi}{4} \times l_i \times w_i,$$

where l_i and w_i represent length and width of wound surface at i th day post-wounding.

2.7c Haematoxylin and eosin (H&E) staining: On 14th day, animals were anaesthetized for tissue sample collection. Tissue samples were immediately fixed by immersion in 10% formaldehyde solution, followed by routine histological processing with paraffin embedding. Histological study was performed at the Department of Anapathological Children's Hospital 1, Ho Chi Minh City, Viet Nam.

2.7d Statistical analysis: Data are represented as means ± standard error ($n = 3$). Two way analysis of variance (SPSS software) was used for the analysis of cytotoxicity on fibroblast cells and wound contraction. A p -value of < 0.05 was accepted as a statistically significant difference.

3. Results and discussion

3.1 Characterization of copolymers

Despite an attractive biomaterial for not only tissue regeneration, but also drug-delivery system, raw gelatin shows the

stiffness in terms of hydrogel due to low mechanical strength. The most common way of approaching this problem is modification of gelatin backbone through a grafting method. In this study, gelatin was modified with Pluronic F127 to prepare hydrogels with good biodegradation and biocompatibility for wound dressing. Pluronic F127 has hydroxyl groups, was activated with p-NPC (4-nitrophenyl chloroformate) with two steps as in the previous report [21] resulting in the formation of a NPC-remaining moiety of NPC-P-OH, which reacted with the amino group on gelatin; consequently, PG was obtained.

The structure of the grafted polymer was verified via FT-IR spectroscopy (figure 2a) by a comparison of absorption peaks in the infrared spectrum between raw gelatin, NPC-P-OH as well as PG copolymer. Obviously, a wide peak in the range of $3500\text{--}3100\text{ cm}^{-1}$, respectively attributed to the stretching vibrations of N-H and O-H, shows a strong intensity in gelatin whereas the intensity is lower in Pluronic F127 and NPC-P-OH. Compared to pure gelatin and NPC-P-OH, the stretching vibration peak of PG copolymer in the range of $3500\text{--}3100\text{ cm}^{-1}$ shifted to the lower wavenumber from ~ 3400 to $\sim 3350\text{ cm}^{-1}$ with the increase of Pluronic F127 in the grafting reaction. In addition, the C=O stretching vibration peak of the amide in gelatin shifted from 1647 to above 1652 cm^{-1} in the PG sample. All these changes indicated that new bonds were formed between gelatin and Pluronic F127.

To provide a strong evidence for the formation of PG copolymer, $^1\text{H-NMR}$ spectrum of PG was obtained. In the spectrum, the resonance peak at $7.23\text{--}7.29\text{ ppm}$ indicated aromatic protons of phenylalanine and other typical protons of amino acids in gelatin as noted in figure 2b. Moreover, the signal at 3.0 ppm assigned to the primary amino group shifted from its original position to 2.8 ppm in the PG copolymer indicating the presence of the urethane bond. Furthermore, the exhibiting proton signals of the pluronic ($-\text{CH}_3$ of PPO at 1.08 ppm and $-\text{CH}_2$ of PEO at 3.6 ppm) confirmed that PG copolymer was successfully prepared.

3.2 Thermo-reversible behaviour

Thermoreversible PG copolymer for topical delivery of Cur should be gel at skin and body temperatures ($32\text{--}36^\circ\text{C}$), while existing as a solution at room temperature. The thermosensitive behaviour of PG copolymer with various amounts of pluronic used in the grafted reaction (PG 1:05, PG 1:10, PG 1:15, PG 1:20) and concentration was investigated by the inverted test tube method (visual observation of mobility) following the increase of temperature in the range of $4\text{--}50^\circ\text{C}$ in the same manner as in the previous study [21]; presented in figure 3a. Phase diagram of sol-gel transition behaviour in figure 3b indicates that increasing the F127 concentration led to the sol-gel transforming temperature following the properties of pluronic rather than gelatin properties, which was in agreement with previous reports [21]. PG at ratio 1:5 was gel-like phase at 4°C while in solution phases at higher temperature ($>30^\circ\text{C}$), corresponding to the property of gelatin.

Figure 3. (a) The visual observation of mobility of nCur-GP 1:15 (15% wt/v) at different temperatures, left side shows sol phase whereas gel-like phase is seen on the right. (b) Phase diagram of sol-gel transition behaviour of PG copolymer solution built by the inverted tube method.

On increasing the content of pluronic in the grafted copolymer (PG 1:10, PG 1:15 and PG 1:20), samples were in the solution phase at lower temperatures, but formed transparent hydrogels at higher temperatures following the thermal property of pluronic. For PG 1:10 sample, the gelation occurred when its concentration was higher 12.5 wt/v% at 30°C , however, its physical property was weak. At the same temperature, PG 1:15 and PG 1:20 showed gelation at lower concentration of PG copolymer (around 10 w/v%) and the formed gels were highly stable at 15 w/v% of PG which could be used for further studies. Furthermore, the thermo-reversible characteristic of PG hydrogel can be applied easily to dissolve for the use in therapeutic agents by cooling the composite hydrogels below their gelation temperature, which is attractive for fundamental tissue regeneration.

3.3 Biodegradation test

To characterize water absorption and stability of PG copolymer, behaviour of the three samples PG 1:15, PG 1:20 and pluronic was tested in PBS buffer of pH 7.4 at 37°C as a function of time. As shown in figure 4, it was found that PG 1:15 attained equilibrium swelling by 7 days while pluronic and PG 1:20 as the same concentration (20 wt/v %) showed dramatically different swelling behaviours without the equilibrium. This trend might be due to the enhancement of hydrogen bonding interactions between F127 and

Figure 4. Biodegradation behaviour of the sample PG 1:15, PG 1:20 and Pluronic F127.

gelatin in PG 1:15 compared to PG 1:20 due to adjusting of the hydrophobic–hydrophilic balance in the system [28]. In the case of examination of sample weight lost during

immersing in PBS (pH = 7.4) at 37°C, PG 1:15 absorbed much more PBS resulting in dry weight increase in the first 7 days and maintained their dry weight in the following 5 days. At day 15, the network of PG 1:15 was broken and the liquid-like content flowed out, causing a dramatically decreased dry weight. Pluronic and PG 1:20 exhibited the weight loss from the initial experiment time. However, pluronic gel was rapidly degraded within 2 days whereas PG 1:20 required 12 days to dissolve completely. This behaviour may be because of two reasons. The conjugation of gelatin molecule with pluronic increased many side chains of the grafted copolymers and resulted in entangled polymer chains enhancing the stability of the hydrogel against degradation and higher swelling lead to a slow mass erosion. These results demonstrated that the PG 1:15 gel had excellent stability in physiologically relevant conditions.

3.4 Characterization of nCur-loaded thermogel

Several reports indicated that nano-scaled Cur could enhance cellular absorption and biodistribution of the hydrophobic

Figure 5. TEM image of nCur dispersed in PG 1:15 with (a) 0.5, (b) 5 and (c) 10% (wt/wt) Cur.

Figure 6. Particle size distribution of nCur dispersed in PG 1:15 with (a) 0.5, (b) 5 and (c) 10% (wt/wt) Cur.

Figure 7. Release profile of nCur in PG gel in PBS (pH = 7.4) at 35°C.

molecule [29]. Therefore, ultrasonication, milling, using surfactant, etc. are attractive methods for nCur processing. In this study, nCur was formulated in the PG copolymer solution along with assisted ultrasonication. Cur powder was dissolved in ethanol and then added drop-wise into PG solution and treated in an Ultrasonic device UP200Ht. After sonication, Cur nanocrystals were separated from solution by centrifugation and re-suspended in DI water for further characterization.

It is more interesting that the nanosuspension solution could form nanocomposite hydrogel on being warmed up (figure 3a). The nCur could form in the PG copolymer solution as concentrated and the PG copolymer contributes

to stability of the nCur in the hydrophobic domain of PG [30]. Moreover, zeta potential measurements showed the positively charged PG copolymer and the negatively charged nCur (data not shown here) which played a significant role in gelatin for enhancing the stability of nCur due its electrostatic interaction. To minimize the Cur/PG complex particle size, the size distribution and morphology of the Cur-loaded PG copolymer, various initial Cur concentration were obtained by TEM (figure 5) and DLS (figure 6), respectively. The size of the round-shaped nCur significantly increases corresponding to an increase in Cur concentration at the initial solution. DLS reveals hydrodynamic diameter of nanoparticles as the function of concentration, which is a higher concentration of Cur loaded in the same PG in copolymer solution and a larger size diameter of formed nanoparticles obtained such as 1.5 ± 0.5 nm (0.5 wt/wt%), 7 ± 0.5 nm (5 wt/wt%) and 16 ± 3.2 nm (10 wt/wt%). However, all the TEM images show the nCur-PG morphological appearance of these nanoparticles which are relatively uniform and spherical in shape despite the changes in the concentration of the loaded Cur.

The drug release profile is of great importance for practical drug-delivery applications of the proposed hydrogel dressing. The aim of this study is to investigate whether nCur-PG hydrogel could be used in wound dressing; thus, *in vitro* drug release studies were conducted via the direct dispersion method at pH 7.4 in PBS buffer and the release pattern as a function of time is shown in figure 7. The graph elucidates the mediated nCur release trend over time, providing the potential matrix for drug delivery at the site administration.

Figure 8. Cell density in the incubated samples.

Figure 9. Macroscopic image of wound surface in animal model at 2, 8 and 14 days post treatment (a) and the wound contracted area over treatment time (b). The error bar was presented by $\pm$ SE. * is assigned to the statistic difference with $p < 0.05$ while ns is seen for non-significant difference ($p > 0.05$).

3.5 Biocompatibility of nCur-PG

To evaluate the merits of hydrogel, cytotoxicity test was performed to determine *in vitro* biocompatibility of the PG hydrogel with and without nCur using a cell-based direct contact test [31]. The time-dependent effects of PG hydrogel with/without nCur on cell viability at 37°C are depicted in figure 8. All formulations presented no harmful effect of the derivative on fibroblast cells after 48 h incubation indicating the good cytocompatibility of hydrogels. The statistical analysis revealed that fibroblast cells had an increased metabolic activity on the PG hydrogel and nCur-PG hydrogel with

the increase of culture time, indicating that the two kinds of PG hydrogels were able to support cell proliferation. The highest cell density was in PG containing nCur ($n=3$, $p<0.01$), following PG samples ($n=3$, $p<0.05$). In general, gelatin-based hydrogel could stimulate fibroblast adhesion and proliferation due to cell-binding sequences of Arg-Gly-Asp. Thus, the density of fibroblast cell on PG was greater than control samples. In case of nCur-PG, together with the help of excellent cell-response features of gelatin, Cur is reported as a promoting agent for fibroblast cell proliferation, epithelial regeneration and migration [32–34]. This may be due to the Cur application on wound healing.

Figure 10. Histology of (a) normal tissue, (b) burn-damaged tissue and wounds at 14 days postwounding: (c) non-treatment, (d) Biafine, (e) covered with PG gel and (f) nCur-PG.

3.6 Burn healing evaluation

Figure 9 indicates that the PG-treated wound exhibited a faster wound healing rate than that of control, while slower than the rates observed in nCur-PG and commercial dressings. The nCur-PG model described that wound recovery was faster than other groups. Macroscopically, the wounds were almost healed for 10 days. On days 2–8, wound treated with Gel-F127-nCur exhibited a significant difference ($p < 0.05$) in the percent wound contraction as compared to other groups. Wound treated with Gel-F127-nCur for 10–14 days showed no significant difference in the percentage of wound contraction when compared to Gel-F127. This could be derived from faster wound-healing effect of two hydrogel materials leading to higher wound contraction and small diameter of wound beds, so it is hard to measure and indicate a significant difference. However, on day 14, only the group treated with Gel-F127-nCur exhibited new hair at the centre of the wound. More interestingly, in treated group with gel-F127-nCur, the colour at the healing area was the same as the colour of normal skin. While in non-treatment model, all wounds are epithelialized and a raised hypertrophic scar was visible. No hair on wound surface or hypertrophic scar was observed in the PG gel and commercial product-treated models. These results suggest that the application of nCur will accelerate the wound-healing process. This also indicated that the

ability of scar formation reduced significantly. It was marked by wound area reduction and wound recovery as seen in figure 9.

Microscopic images of H&E stained tissue sections are displayed in figure 10. In comparison with normal tissue, tissue sample with a second, degree burn was observed deep into dermis with destroyed cellular structure and tissue structure as seen in figure 10a and b. After 14 days of healing process, the obvious increment in the number of hair follicles was in groups PG gel, nCur-PG and Biafine commercial product compared to the non-treatment models. Only group nCur-PG showed the development of hair sheath, corresponding with the appearance of hair on wound surface. Moreover, in the group, the sebaceous glands were regenerated not only at the edge of the wound but also in the centre, whereas the sebaceous glands regenerated only at the edge of the wound in commercial drug and PG hydrogel-treated models. In addition, re-epithelialization was complete and a thick layer of granulation tissue was observed in three treated groups. In addition, only nCur-PG-treated group exhibited the presence of stratum corneum and stratum granulosum. Most dramatically, tissue reconstruction in Gel-F127-nCur-treated models is the same as the arrangement in the normal skin. In some additional experiments, Masson's trichrome (Tri) staining of the samples also showed that Gel-F127-nCur-treated has more compact and denser collagen alignment (data not shown here).

Based on H&E and Tri results, we suggest that by using nCur-PG, second-degree burn wounds can be healed not only at surface structures, but also in the critical barrier function of skin.

The positive efficacy of the nCur-PG on burn-healing process and regeneration of its functional tissue could be contributed synergistically by gelatin-based hydrogel and the encapsulated nCur in the nCur-PG [34–36].

4. Conclusions

We successfully synthesized the thermosensitive PG copolymer which served as a dispersant to produce small size and high content of nCur (<20 nm and 10 wt/wt%, respectively). The nCur-dispersed PG copolymer solution could form a nanocomposite hydrogel at the physiological temperature (around 35°C). Sustainable release profile of Cur from the hydrogel matrix provided a desirable vehicle to control delivery of Cur at a suitable concentration for enhancing wound healing (low concentration of encapsulated Cur). These obtained results could pave way for applications of the thermosensitive nCur-loaded platform in biomedical field.

Acknowledgements

This work was financially supported by Tra Vinh University under grant number 1434/HD.DHTV-KHCN and Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104.02-2017.60.

References

- [1] Thangapazham R L, Sharad S and Maheshwari R K 2016 *Adv. Wound Care* textbf5 230
- [2] Tran N Q, Joung Y K, Lih E and Park K D 2011 *Biomacromolecules* **12** 2872
- [3] Aggarwal B B and Harikumar K B 2009 *Int. J. Biochem. Cell Biol.* **41** 40
- [4] Anand P, Kunnumakkara A B, Newmanand R A, Aggarwal B B 2007 *Mol. Pharm.* **4** 807
- [5] Cheppudira B, Fowler M, McGhee L, Greer A, Mares A, Petz L *et al* 2013 *Expert Opin. Invest. Drugs* **22** 1295
- [6] Emiroglu G, Coskun Z O, Kalkan Y, Erdivanli O C, Tumkaya L, Terzi S *et al* 2017 *J. Evid. Based Complementary Altern. Med.* **2017** ID 9452392
- [7] Sharma R A, Steward W P and Gescher A J 2007 *Adv. Exp. Med. Biol.* **595** 453
- [8] Yuvarani I, Kumar S S, Venkatesan J, Kim S K and Sudha P N 2012 *J. Biomater. Tissue Eng.* **2** 54
- [9] Gong C Y, Wu Q J, Wang Y J, Zhang D D, Luo F, Zhao X, Wei Y Q and Qian Z Y 2013 *Biomaterials* **34** 6377
- [10] Sharma M, Sahu K, Singh S P and Jain B 2018 *Artif. Cells Nanomed. Biotechnol.* **28** 1009
- [11] Akbik D, Ghadiri M, Chrzanowski W and Rohanizadeh R 2014 *Life Sci.* **116** 1
- [12] Merrell J G, McLaughlin S W, Tie L, Laurencin C T, Chen A F and Nair L S 2009 *Clin. Exp. Pharmacol. Physiol.* **36** 1149
- [13] Carvalho D M, Takeuchi K P and Geraldine R M and Moura C J 2015 *Food Sci. Technol.* **35** 115
- [14] Lin C C, Lin H Y, Chen H C, Yu M W and Lee M H 2009 *Food Chem.* **116** 923
- [15] Kakkar V, Singh S, Singla D and Kaur IP 2011 *Mol. Nutr. Food Res.* **55** 495
- [16] Le P N, Huynh C K and Tran N Q 2018 *Mater. Sci. Eng. C* **92** 1016
- [17] Tran N Q, Choi J H, Bae J W, Choi J W, Joung Y K and Park K D 2011 *Macromol. Res.* **19** 180
- [18] Bonacucina G, Cespi M, Mencarelli G, Giorgioni G and Palmieri G F 2011 *Polymers* **3** 779
- [19] Wang K and Han Z C 2017 *J. Control. Release* **268** 212
- [20] Tong N A N, Nguyen T P, Nguyen C K and Tran N Q 2016 *J. Biomater. Sci. Polym. Ed.* **27** 709
- [21] Nguyen T T C, Nguyen T H, Tran N Q and Nguyen C K 2017 *Mater. Sci. Eng. C: Mater. Biol. Appl.* **70** 992
- [22] Davidenko N, Schuster C F, Bax D V, Farndale R W, Hamaia S, Best S M and Cameron R E 2016 *J. Mater. Sci. Mater. Med.* **27** 148
- [23] Van T D, Tran N Q, Nguyen D H, Nguyen C K, Tran D L and Nguyen P T 2016 *J. Electron. Mater.* **45** 2415
- [24] Balakrishnan B, Mohanty M, Umashankar P R and Jayakrishnan A 2005 *Biomaterials* **26** 6335
- [25] Nguyen T B T, Dang L H, Nguyen T T T, Tran D L, Nguyen D H, Nguyen V T *et al* 2016 *Green Process. Synth.* **5** 511
- [26] Higuchi A, Sugiyama K, Yoon B, Sakurai M, Hara M, Sumita M *et al* 2003 *Biomaterials* **24** 3235
- [27] Jia W J, Liu J G, Zhang Y D and Wang J W 2007 *J. Drug Target.* **15** 140
- [28] Southall N T, Dill K A and Haymet A D J 2002 *J. Phys. Chem. B* **106** 521
- [29] Feng S S 2004 *Expert Rev. Med. Devices* **1** 115
- [30] Yallapu M M, Jaggi M and Chauhan S C 2012 *Drug Discov. Today* **17** 71
- [31] Li W, Zhou J and Xu Y 2015 *Biomed. Rep.* **3** 617
- [32] Lee S M, Chiang S H, Wang H Y, Wu P S and Lin C C 2015 *Biosci. Biotechnol. Biochem.* **79** 247
- [33] Dang L H, Nguyen T H, Tran L B H, Doan N V and Tran N Q 2018 *J. Healthc. Eng.* **5754890** 14
- [34] Kang J Y, Huang H and Zhu F Q 2009 *Zhongguo Zhong Xi Yi Jie He Za Zhi* **29** 1100
- [35] Vatankhah E, Prabhakaran M P, Jin G, Mobarakeh L G and Ramakrishna S 2014 *J. Biomater. Appl.* **28** 909
- [36] Pandima Devi M, Sekar M, Chamundeswari M, Moorthy A, Krithiga G, Selva M N and Sastry T P 2012 *Bull. Mater. Sci.* **5** 1157