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NANOCURCUMIN AND CHITOSAN-PLURONIC F127-BASED HYDROGEL FOR 3RD DEGREE BURN TREATMENT

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Abstract. Burn is one of the popular accidents today and usually leaves serious physical and mental damage. Previously, burn was considered acute wounds, but now it is evolution into chronic wounds if inadequately managed. Up to now, there have been abundant of natural and synthetic products for burn healing. In the study, we fabricated a thermosensitive nanocomposite hydrogel, which incorporated dual active curcumin and chitosan. Beside of a well-known characteristic of chitosan for wound healing, curcumin has been a lot of interest in burn wound healing application due to ability in depleting the action of oxidative radicals and stimulation of fibroblast cells. In order to enhance the therapeutic efficacy of curcumin, we introduced a new method to synthesize nanocurcumin in the thermosensitive chitosan-g-Pluronic F127 copolymer solution under ultrasonication. The rheology of aqueous solutions of this material was studied as a function of temperature. The solutions of this material undergo a transition to a gel at higher temperature, above which a complex rheological behavior is observed. In addition, a minimum inhibitory concentration of this material was determined for a variety of bacterial and was compared to that of curcumin. It was found that the aqueous dispersion of this material was much more effective than curcumin against both positive and negative gram bacterial. In the third degree burn models, the nCur-CP hydrogel performed a higher burn healing rate as compared to Silvirin-treated burn. These data suggest that the nanocomposite hydrogel may be a great potential for burn treatment.

Keywords: burn healing, curcumin, nanocomposite hydrogel, chitosan, pluronic F127.

Classification numbers: 1.2.6; 2.9.3.

1. INTRODUCTION

The desire to restore skin after being damaged remains a major target of tissue regeneration technology [1, 2]. Nowadays, the substances derived from nature that used to treat and improve human health are increasingly being concerned. In some serious damage cases such as third degree burns it is difficult to recover as original. One of the factors affecting the healing process is the appearance of oxidative free radicals [3, 4]. The reduction of free radicals increases the ability to heal wounds [5, 6]. Curcumin (Cur; 1.7-bis (4-hydroxy-3-methoxyphenyl)-1.6-hepadiene-3.5-dione) is a natural substance found in turmeric (Curcuma longa). It is known as an antioxidant and anti-inflammatory stimulating the growth of fibroblasts [7, 8]. Curcumin has great potential in medical applications, however it is of poor solubility so its application is limited [9, 10]. To enhance the therapeutic effect of curcumin, we developed the formula of injectable nanocomposite hydrogel, which has brought much hope regarding the use of curcumin in the medical field [11, 12, 13].

Pluronic F-127 is a thermosensitive hydrogel capable of dissolving in water and has low toxic properties. It is one of the pluronics belongs to Food and Drug Administration (FDA) for clinical [14]. For clinical application the Pluronic F127 is capable to be dissolved well, biologically compatible (Pluronic F127 as a Cell Encapsulation Material: Utilization of Membrane-Stabilizing Agents) and sensitive to heat [15, 16]. It is considered as a material for loading drug. However, it is possible to combine F127 with chitosan, to increase bio-compatibility and increase antibacterial activity. Chitosan is a natural polymer widely used in the medical field by bio-compatibility, biodegradable, hemostatic activity, and their antibacterial properties [17, 18, 19]. Therefore, chitosan could be used as a factor against bacterial infiltration [20]. From the results of the previous researches, we have conducted the further evaluation of the copolymer-based hydrogel encapsulated nanocurcumin in the treatment of third degree burn [11]. Regarding to synergic effects of nanocurcumin and chitosan-based hydrogel, the extensive study could be expected to pave a way for exploiting the injectable nanocomposite hydrogel in clinical application.

2. MATERIALS AND METHODOLOGY

2.1. Material

The hydrogel nCur-CP was synthesized following the procedures in our previous study [11]. The chemicals and solvents in this study were purchased from Sigma (USA) or Schalaurs (Spain). Male white mice (scientific name: Musmusculus var. Albino), of weight of about 35 g was offered from the Pasteur Institute in Ho Chi Minh City.

2.2. Storage study

The curcumin in absolute ethanol was added drop-wise into CP solution under ultrasonication process as shown in Figure 1. Then ethanol was evaporated by the rotary evaporator to obtain an nCur-loaded CP nanocomposite material which could be dissolved in cold distilling water and then form a nanocomposite hydrogel by increasing the solution temperature. The sol-gel transition temperature of the sample was tested. The stability of nanocurcumin-loaded nCur-CP samples was evaluated at time interval of 0, 1, 4 and 24 weeks. These samples were stored in two different temperature conditions, at cold temperatures of 4-8

^{o}C and at room temperature (RT). In the specified period, the product was taken out to evaluate stability of the nanocurcumin in hydrogel.

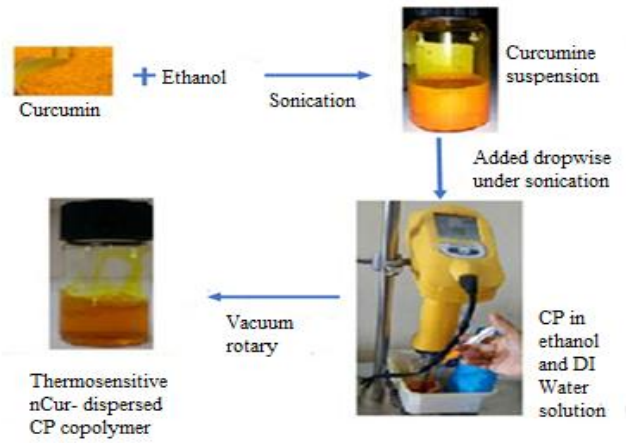

Figure 1. Prepared nanocurcumin loaded chitosan-g-pluronic F127.

2.3. *In vitro* evaluation of the injectable nCur-CP materials

2.3.1. Antibacterial activity

The types of bacteria used to evaluate the antibacterial activity of curcumin were *Escherichia coli* (ATCC8739), *Salmonella typhimurium* (ATCC 14028), *Pseudomonas aeruginosa* (ATCC27853) and *Staphylococcus aureus* (ATCC 6538). The Kierby-Bauer disk diffusion method used in this experiment was obtained from Department of Biochemistry, Faculty of Biology and Biotechnology, University of Science, Vietnam National University, Ho Chi Minh city. These bacteria are cultured in nutrient agar before carrying out the tests. The bacteria after culture were diluted in sterile LB solution and adjusted to a number of bacteria in the colony forming (unit: cfu/mL) by a UV spectrophotometer at 660 nm. In this experiment, we have used 4 different variables including hydrogel, nCur-CP, curcumin solutions and antibiotics chloramphenicol as the control. The concentration of curcumin used in this testing was equivalent to the concentration of curcumin in nCur-CP composite.

Raw curcumin solution was prepared by dissolving in DMSO, the remaining variables were dissolved in water. For each individual agar plate were added with varying concentrations of curcumin (0.08-4 mg/ml), hydrogel (0.75-3.7 mg/mL) and nanocomposite solutions. A plate of agar added DMSO was used as the control plates. Minimum Inhibitory Concentration (MICs) was the lowest concentration that completely inhibits the growth of bacteria.

2.3.2. Cell culture

In this study, the Human Foreskin fibroblasts (HFF-1; SCRC-1041TM; USA) were used to evaluate the toxicity and proliferation of living cells. The HFF1 cells were cultured in standard culture medium (Dulbecco's 10 % bovine fetal modified Eagle medium serum, 100 U/ml penicillin G, and 100 µg/ml streptomycin). The culture medium was replaced twice a day. Cell growth (HFF-1) was evaluated on three groups: nCur-CP, hydrogel and 2D tissue culture

control. HFF1- cell proliferation was measured with Alamar Blue (Sigma Aldrich, St. Louis, MO, USA) and fluorescence microscope (TE2000, Nikon, Seoul, Korea).

2.4. *In vivo* evaluation of burn wound healing in animal model

The 3rd-degree burn wounds on mice were conducted at the Laboratory of the Department of Physiology and Animal Biotechnology, University of Science, Vietnam National University-Ho Chi Minh City. Mice were kept stable for 4 days before carrying out experiments. Mice were weighed and anesthetized with ketamine intraperitoneal (100 mg/ml) and xylazine (20 mg/ml) with dosage of 0.2 ml / 100 g body weight before shaving the dorsal skin of mice. Sterilize the shaved skin with 1 % povidine before proceeding to burn. To create the 3rd-degree burn, a cylindrical shaped stainless steel cup with a diameter of 1 cm, weight 114 g was heated in a hot water (100 degrees). Then steel bar was placed over the cleaned skin and held at different intervals (5s, 10s and 20s). After 2 or 3 hours, these skins were collected and kept in formaldehyde 10 % about 24 hours for H&E dyeing.

In this study, animals were divided into three groups, each group consisted of three mice:

Group I: Control (non-treatment),

Group II: Using commercial drugs (Silvirin),

Group III: Using nCur-CP.

The 3rd-degree burn was studied within 22 days, the wound was measured twice a day. After 7, 14, 22 days treatment, skin samples were collected and stored in formaldehyde 10% to dyeing H & E and using ImageJver 1.41 to evaluate the effect of treatment.

2.5. Statistical analysis

The data collected from the experiments were analyzed statistically by using one-way ANOVA for particle size, the test of antibiotics or curcumin content, etc. The data related to the comparison between the two independent variables were evaluated by using two-way ANOVA for cell viability and closed wound surface. They are considered statistically significant with the value $p < 0.05$.

3. RESULTS

3.1. The sol-gel transition temperature

Rheological measurement was used to determine the sol - gel transition temperature of the nanocomposite hydrogel. The point was observed on the change of storage modulus G' and loss modulus G" at temperature ranging from 4 °C to 45 °C with frequency and amplitude fixed. The operation defined here as the temperature at which the storage modulus G' and loss modulus G" are equal. As shown in Figure 2, at low temperature G">>G' it means that the hydrogel is in sol state. At 30 ^{o}C both G’ and G’’ rise together, when temperature reach 35 ^{o}C the two values of G’ and G’’ are nearly equal. This indicates that the sol - gel transition temperature (Tgel) of the hydrogel is about 35 °C. This Tgel is closer to human body temperature (about 37 °C). Therefore, injectable nanocomposite hydrogel has great potential to develop into a drug delivery system applicable for wound healing [11].

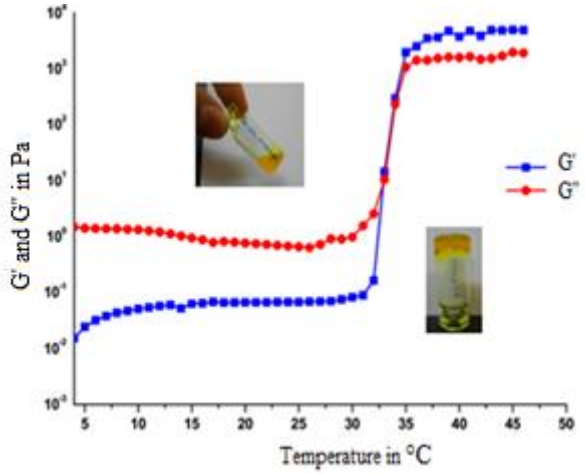

Figure 2. Rheology of nCur-loaded CP hydrogel (CS: F127 = 1:15) as dependent on temperature.

After 4 and 24 weeks of storage, the storage samples were collected to test stability of the nanocurcumin. The results show that there is no change in color of all nanocomposite hydrogel samples as well as size distribution of the nanoparticles as stored at cool or room temperature for 4 weeks as shown in Figure 3. Significant increase in the particle size in RT condition and increase slightly in cool condition after 24 weeks were observed.

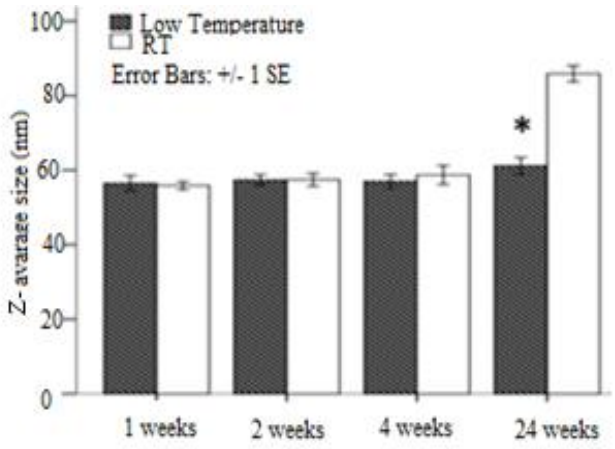

Figure 3. Size of curcumin nanoparticles in the CP copolymer phase at low temperature and ambient conditions (RT).

3.2. *In vitro* evaluation of nCur-CP nanocomposite hydrogel

3.2.1. Anti-biotic ability

The zones of growth inhibition were measured after 18 to 24 h incubation at 37 °C for bacteria. The antibacteria activity of materials was determined by the measured diameter of growth inhibitory zones as indicated in Figure 4, and if the value is equal or less than 6 mm which means that no antibacteria activity occurred. The antibacterial activity of the antibiotics chloramphenicol was the same for all four bacterial groups. nCur-CP and hydrogel were more effective against *P. aeruginosa*, *E. coli* and *S. aureus* than against *S. typhi.* Raw curcumin

samples showed the most effective against *S. aureus* while less effective against *E. coli*, *P. aeruginosa* and *S. typhi*. In addition, by using ANOVA one way for each bacterial group, the nCur- CP had maximum antibacterial efficiency in all the tests ($p < 0.05$).

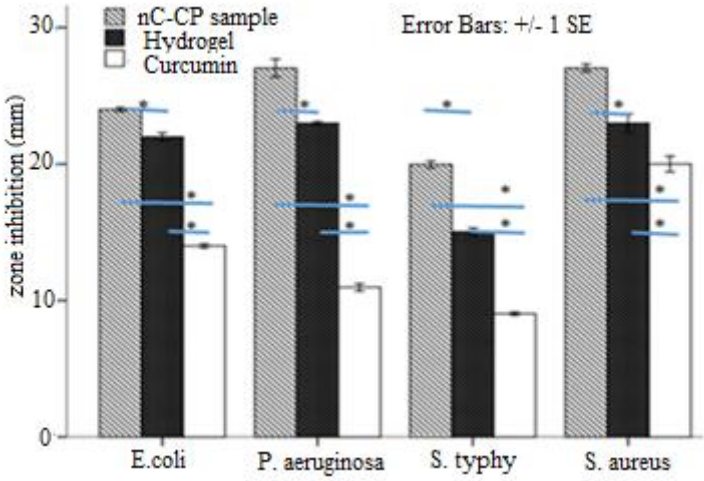

Figure 4. Zone of inhibition of hydrogel, nCur-CP and raw curcumin solutions against 4 bacteria strains: *Escherichia coli*; *Pseudomonas aeruginosa; Salmonella typhimurium* and *Staphylococcus aureus.*

Table 1 has shown that the minimum inhibitory concentration (MIC) of raw curcumin for *E. coli, S. typhimurium, P. aeruginosawas and S. aureus* were 1.6, 4, 2 and 0.96 (ppm), respectively; and the MIC of hydrogel in turn is 18.75, 37.5, 9 and 15 (mg/ml). From the results it is found that the raw curcumin had antibacteial activity better than that of hydrogel. However, MIC of nCur-CP was lower than both MIC of raw curcumin and hydrogel. The effect could be contributed by a synergically active combination of both chitosan hydrogel and curcumin.

Table 1. MIC of raw curcumin, hydrogel and nCur-CP against different microbes.

Organisms	**MIC**		
	Raw curcumin (ppm)	**Hydrogel (mg/ml)**	**nCur-CP (mg/ml)**
E. coli	1.6	18.75	3.0 (0.32 ppm curcumin)
S. typhimurium	4	37.5	4.5 (0.48 ppm curcumin)
P. aeruginosa	2	9.0	0.75 (0.08ppm curcumin)
S. aureus	0.96	15	1.5 (0.16 ppm curcumin)

3.2.2. Biocompatibility test

In this study, the fibroblast cells were used for evaluation because of their involvement in the wound healing [21]. The number of fibroblasts in different environments was shown in Figure 5A. The number of fibroblasts on the nCur-CP sample was greater than all other environments. This could be caused by the presence of small amount of curcumin released into media culture, which could contribute to increase proliferation of fibroblasts [22]. In addition, Figure 5B also confirms that the chitosan hydrogel is well-biocompatible as shown that most of

cells are alive. For the fluorescent staining, living cells are stained with green color and dead cells with red color.

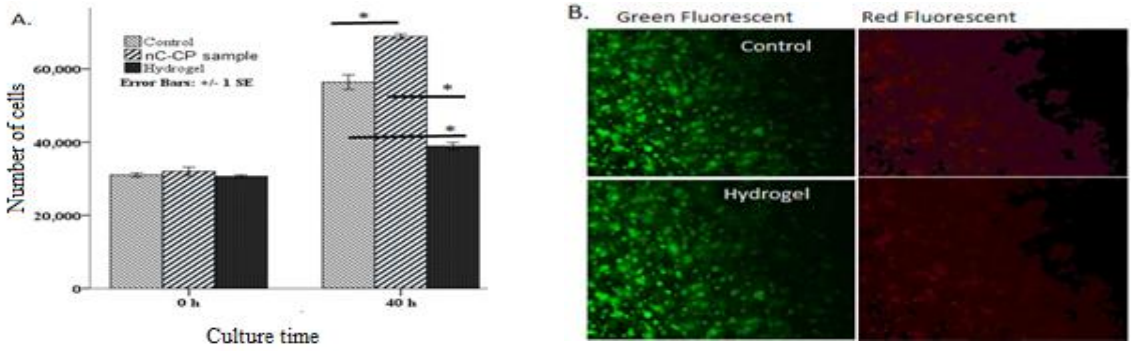

Figure 5. Effect of culture environment on cell proliferation (A) and live/dead staining of cell incubated in the chitosan hydrogel (B).

3.3. In vivo evaluation of third burn wound healing in mice model

Third degree burn model was performed following the protocol at the University of Science. Examination of healing rate and wound contraction were based on the rate of closed wound and histological staining (H&E), respectively. The calculation of closed wound was applied following the formula of Lyman et al, as below:

$$S = \frac{\pi \times R \times D}{4}$$

In that: S_ is area of the wound; R _ width of the wound and D _length of the wound.

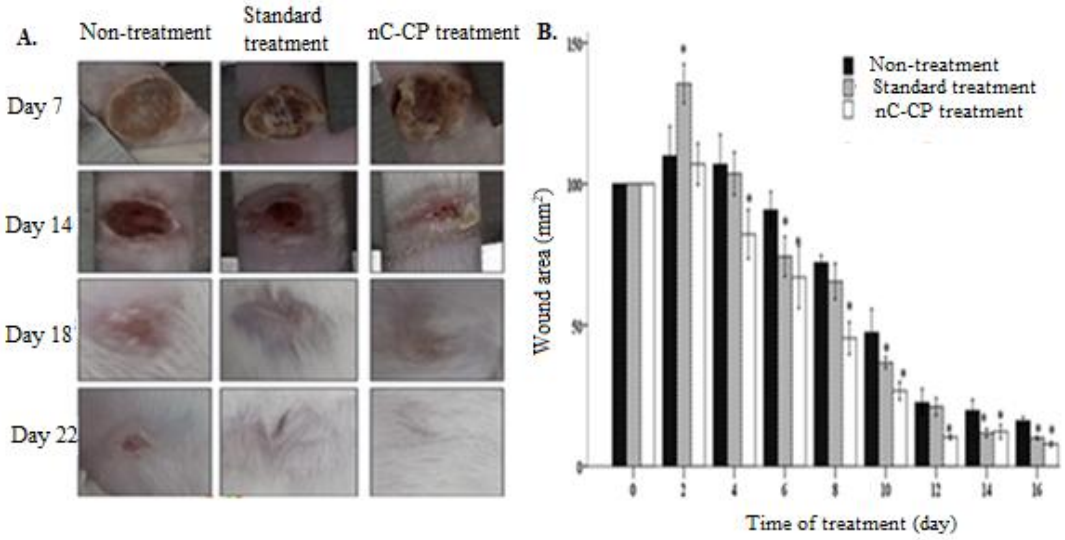

Figure 6. Digital photographical images of morphological wounds (A), and wound contraction of treated and non-treated models (B).

After 7 days burned, all three treated groups were found to be not different by the external morphology. From day 7 to day 12, the morphology of 3 treatment groups was changed. The

area of the wound had significant changes, especially in group treatment by nCur-CP that decreased from 82.2 ± 17.35 $mm^2$to 12.2 ± 4.9 mm^2 ($p < 0.05$). After 20 days of Silvirin and nCur-CP treatment, wounds were healed completely. The new blood vessels were appeared in the nCur-CP treatment group while less in the group treated with Silvirin. Epithelial formation process in treatment group with nCur-CP was faster than that treated with Silvirin ($p < 0.05$). In addition, the appearance of the collagen fibers in the nCur-CP treatment group was earlier than treatment group with Silvirin. Growth of hair follicle in the nCur-CP-treated wound was more than that of Silviri treatment. Besides, the epidermis layer of the nCur-CP and Silvirin-treated models were thinner than the non-treated group, and the skin became normal (Figure 6A) as for both morphology and histology. From the above results it can be concluded that the burn wounds treated with nCur-CP had better efficiency than the other groups.

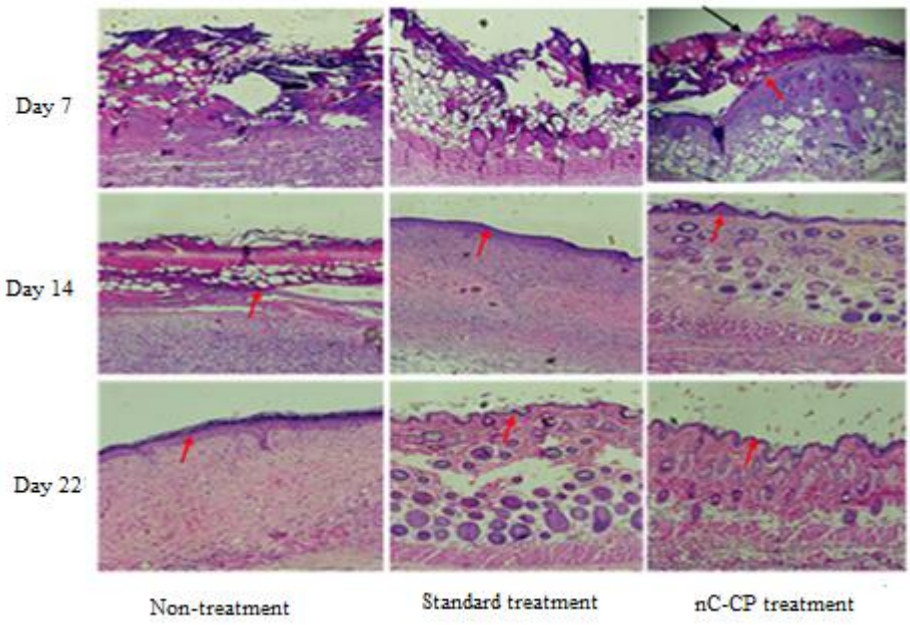

Figure 7. Histological examination of the healing process at different treatment groups (7, 14 and 22 days post wounding). All the images were observed at 10x magnification and scale bar = 500. Black arrows and red arrows used to point out the crush and re-epithelialized layer, respectively.

4. CONCLUSION

Injectable nanocurcumin formulated chitosan-g-pluronic hydrogel based on curcumin and thermosensitive pluronic F127-grafted chitosan copolymers were prepared and efficiency-evaluated on the 3rd-degree burn model. In addition, assessments of biocompatibility, antibacterial activity and treatment efficacy on 3rd-degree burn model were performed. The results have shown the positive efficiency of the nCur-CP nanocomposite in healing 3rd burn injury. The material has great potential and is useful in human health care.

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REFERENCES

1. Wei X., Senanayake T. H., Warren G. and Vinogradov S. V. - Hyaluronic acid-based nanogel-drug conjugates with enhanced anticancer activity designed for targeting of CD44-positive and drug-resistant tumors, Bioconjug Chem **24** (24) (2013) 658–668.
2. Gerritsen M., Jansen J. A., Kros A., Nolte R. J. and Lutterman J. A. - Performance of subcutaneously implanted glucose sensors: a review, Journal of Investigative Surgery **11** (1998) 163.
3. Guo S. and DiPietro L. A. - Factors Affecting Wound Healing, J. Dent. Res. **89** (3) (2010) 219-29.
4. Thangapazham R. L., Sharad S. and Maheshwari R. K. - Phytochemicals in eound healing, Adv Wound Care (New Rochelle) **5** (5) (2016) 230-241.
5. Thangavel P., Ramachandran B., Chakraborty S., Kannan R., Lonchin S. and Muthuvijayan V. - Accelerated healing of diabetic wounds treated with l-glutamic acid loaded hydrogels through enhanced collagen deposition and angiogenesis: An in vivo study, Sci Rep **7** (2017) 10701.
6. Nirwana I., Rachmadi P. and Rianti D. - Potential of pomegranate fruit extract (Punica granatum Linn.) to increase vascular endothelial growth factor and platelet-derived growth factor expressions on the post-tooth extraction wound of Cavia cobaya, Veterinary world **10** (8) (2017) 999.
7. Aggarwal B. B., Yuan W., Li S. and Gupta S. C. - Curcumin-free turmeric exhibits anti-inflammatory and anticancer activities: Identification of novel components of turmeric, Mol Nutr Food Res **57** (9) (2013) 1529-1542.
8. Tejada S., Manayi A., Daglia M., Seyed F. N., Sureda A., Hajheydari Z., Gortzi O., Pazoki-Toroudi H. and Nabavi S. M. - Wound healing effects of curcumin: a short review, Curr Pharm Biotechnol **17** (11) (2016) 1002-1007.
9. Ghalandarlaki N., Alizadeh A. M. and Ashkani-Esfahani S. - Nanotechnology-Applied curcumin for different diseases therapy, Biomed Res Int **2014** (2014) 394264.
10. Ravichandran R. - Pharmacokinetic study of nanoparticulate curcumin: oral formulation for enhanced bioavaibility, Journal of Biomaterials and Nanobiotechnology **4** (2013) 291-299.
11. Nguyen T. B. T., Dang L. H., Nguyen T. T. T., Tran D. L., Nguyen D. H., Nguyen V. T., Nguyen C. K., Nguyen T. H., Le V. T. and Tran N. Q. - Green processing of thermosensitive nanocurcumin-encapsulated chitosan hydrogel towards biomedical application, Green Processing and Synthesis **5** (6) (2016) 511-520.
12. Tran N. Q., Joung Y. K., Lih E., Park K. M., and Park K. D. - Supramolecular hydrogels exhibiting fast in situ gel forming and adjustable degradation properties, Biomacromolecules **11** (3) (2010) 617-625.
13. Dabiri G., Damstetter E. and Phillips T. - Choosing a Wound Dressing Based on Common Wound Characteristics, Adv Wound Care (New Rochelle) **5** (1) (2016) 32-41.
14. Kant V., Gopal A., Kumar D., Gopalkrishnan A., Pathak N. N., Kurade N. P., Tandan S. K., and Kumar D. -Topical pluronic F-127 gel application enhances cutaneous wound healing in rats, Acta Histochem **116** (1) (2014) 5-13.

15. Akash M. S. H. and Rehman K. - Recent progress in biomedical applications of Pluronic (PF127): Pharmaceutical perspectives, Journal of Controlled Release **209** (2015) 120-138.
16. Nguyen D. H., Tran N. Q., Nguyen C. K. - Tetronic-grafted chitosan hydrogel as an injectable and biocompatible scaffold for biomedical applications, Journal of Biomaterials Science, Polymer Edition **24** (14) (2013) 1636-1648.
17. Zhou L. H., Nahm W. K., Badiavas E., Yufit T. and Falanga V. - Slow release iodine preparation and wound healing: in vitro effects consistent with lack of in vivo toxicity in human chronic wounds,Br J Dermatol **146** (3) (2002) 365-374.
18. Tran N. Q., Joung Y. K., Lih E. and Park K. D. – In situ forming and rutin-releaseing chitosan hydrogels as injesctable dressings for dermal wound healing, Biomacromolecules **12** (8) (2011) 2872-2880.
19. Khor E., Wu H., Lim L. Y. and Guo C. M. - Chitin-methacrylate: preparation, characterization and hydrogel formation, Materials (Basel) **4** (10) (2011) 1728-1746.
20. Nguyen T. P. and Tran N. Q. - Preparation and biomineralization of injectable hydrogel composite based chitosan-tetronic and bcp nanoparticles, Advances in Research **7** (2016) 1-8.
21. Darby I. A., Laverdet B., Bonté F. and Desmoulière A. - Fibroblasts and myofibroblasts in wound healing, Clinical, Cosmetic and Investigational Dermatology **7** (2014) 301-311.
22. Dai X., Liu J., Zheng H., Wichmann J., Hopfner U., Sudhop S., Prein C., Shen Y., Machens H. G. and Schilling A. F. - Nano-formulated curcumin accelerates acute wound healing through Dkk-1-mediated fibroblast mobilization and MCP-1-mediated anti-inflammation,NPG Asia Materials **9** (2017) e368.