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Research Article

Curcumin-Loaded Chitosan/Gelatin Composite Sponge for Wound Healing Application

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Three composite sponges were made with 10% of curcumin and by using polymers, namely, chitosan and gelatin with various ratios. The chemical structure and morphology were evaluated by FTIR and SEM. These sponges were evaluated for water absorption capacity, antibacterial activity, *in vitro* drug release, and *in vivo* wound healing studies by excision wound model using rabbits. The *in vivo* study presented a greater wound closure in wounds treated with curcumin-composite sponge than those with composite sponge without curcumin and untreated group. These obtained results showed that combination of curcumin, chitosan and gelatin could improve the wound healing activity in comparison to chitosan, and gelatin without curcumin.

1. Introduction

Medicines derived from plants play an important role in the healthcare of many cultures, both ancient and modern. Curcumin (diferuloylmethane, a polyphenol) is an active agent of the perennial herb *Curcuma longa* (commonly known as turmeric). Curcumin is a naturally occurring polyphenolic phytoconstituent which presents anticancer, antioxidant, anti-inflammatory, hyperlipidemic, antibacterial, wound healing, and hepatoprotective activities [1, 2]. The therapeutic efficacy of curcumin is limited due to its poor aqueous solubility and extensive first pass metabolism [3–5]. Topical formulation of curcumin (curcumin incorporated collagen matrix) was a feasible and productive approach to support dermal wound healing [1]. Therefore, development of novel curcumin formulation and delivery systems is required. The topical delivery of naturally occurring compounds, such as curcumin or catechins, is able to increase its solubility, stability, and pharmacological activities, resulting in improvement of therapeutic effects [6].

Additionally, chitosan, a polysaccharide biopolymer derived from naturally occurring chitin, displays unique polycationic, chelating, and film-forming properties due to the presence of active amino and hydroxyl functional groups. Chitosan also exhibits a number of interesting biological activities, including antimicrobial activity, induced disease resistance in plants, and diverse stimulating or inhibiting activities toward a number of human cell types [7, 8]. Moreover, chitosan can be used to prevent or treat wound and burn infections due to its intrinsic antimicrobial properties and its ability to deliver extrinsic antimicrobial agents to wounds and burns [9]. Additionally, it can accelerate the functions of inflammatory cells, macrophages, and fibroblasts [10, 11]. Chitosan and its derivatives are also used to increase the stability of the drug in which the drugs are loaded in chitosan film or chitosan nanoparticles, resulting in enhancement of drug accumulation [12] and toxicity to cancer cells [13, 14]. Gelatin is also a natural polymer derived from collagen of animal skin and bones. It is biocompatible, hydrophilic, and biodegradable under normal physiological conditions.

Gelatin is known to prevent fluid loss due to exudation, resulting in enhancement of its wound healing properties [15, 16]. Thus, the present study focused on the preparation and evaluation of composite sponge made with two natural polymers: chitosan and gelatin at different proportions. Moreover, curcumin was loaded into the chitosan and gelatin sponge for wound healing application.

2. Materials and Methods

2.1. Materials. Chitosan is extracted from crab shells which was prepared from our lab. The degree of deacetylation of the chitosan was approximately 85%. Curcumin was purchased from Vietnam Institute of Dietary Supplement (Hanoi, Vietnam) and acetic acid and gelatin were purchased from Sigma-Aldrich Chemical Co (USA). All chemicals used in this study were of reagent grade.

2.2. Preparation of Composite Sponge. Chitosan (1.0 g) was dissolved in 1% aqueous acetic acid (100 mL) to form a 1% chitosan solution (w/v) at room temperature. Gelatin (10%) solution was prepared by dissolving gelatin powder (10 g) in 100 mL of deionized water at room temperature. The composite sponges were prepared by dissolving chitosan solution into the gelatin solution with different portions (3 : 1, 1 : 1, and 1 : 3) of chitosan and gelatin, named as SP3, SP2 and SP1, respectively. The resulting solution was vigorously mixed under magnetic stirrer until the opaque aqueous solution was obtained. The solution was sonicated for 5 min for the removal of entrapped air bubbles. Then the air bubble-free solution was poured into separated petridishes and frozen at −40°C for 24 h followed by lyophilization to obtain composite sponge. For curcumin encapsulated in composite sponge, 1 mL of curcumin solution (0.5% w/v in absolute ethanol) was added to opaque aqueous solution. Then the solution was poured into separated petridishes, frozen at −40°C for 24 h, and followed by lyophilization to obtain curcumin composite sponge.

2.3. Characterization of Composite Sponge. The chemical structure and morphology of chitosan/gelatin composite sponges were evaluated using FT-IR (PerkinElmer) and SEM (JSM-5500).

2.4. Water Absorption Test. The water absorption capacity of the composite sponge was determined by incubating the composite sponge in phosphate-buffered saline (PBS) at pH 7.4 at 37°C for 2 h. The wet weight of the swollen sponge was measured immediately after gently blotting with filter paper to remove absorbed water on surface, followed by lyophilization and reweighing. The water content of the sponge was calculated as follows:

$$E_{\text{ad}} = \left[\frac{W_e - W_0}{W_0}\right] \times 100, \quad (1)$$

where E_{ad} is the percentage water adsorption of chitosan-gelatin sponge at equilibrium. W_e and W_0 represent the weight of the chitosan-gelatin sponge at equilibrium and initial weight of the chitosan-gelatin sponge, respectively.

2.5. Weight Loss of Prepared Sponges. The prepared sponges were immersed in PBS buffer solution at 37°C. After 24 h, the sponges were taken out, washed with deionized water, frozen, and lyophilized. The weights of sponges were measured after lyophilization. Weight loss of sponges was calculated through the following equation: $\%W = (W_0 - We)/W_0 \times 100$, where W_0 and W_e are the initial weight of sponge (g) and weight of sponge after lyophilization (g), respectively.

2.6. In Vitro Drug Release. The composite sponges (3 × 3 cm) were dispersed in 20 mL phosphate buffer solution, pH 7.4 at 37°C. All the supernatants were pipetted out periodically and replaced with equivalent volume of fresh phosphate buffer solution. The concentration of released drug was then determined by UV-Vis spectrometer at 420 nm using a standard curve of curcumin in ethanol. The percentage of curcumin released was determined as curcumin release (%) = curcumin released at time t/total curcumin loaded in sponge × 100.

2.7. Evaluation of Antimicrobial Activity. The measurements of the antimicrobial activities of individual chitosan and composite sponge were conducted by agar diffusion method using *Pseudomonas aeruginosa*. The zone of inhibition was determined by placing a definite size of sponge in inoculated solidified nutrient agar medium in a petriplate. Petriplates were incubated for 24 h at 37°C. This was done in triplicate with each film for each organism, and an average diameter of zone of inhibition was noted.

2.8. Cytotoxicity. The cytotoxicity of curcumin sponge was conducted on mouse fibroblast cells (L929) indirect MTT method [17]. The cells were seeded into 96-well microplates at a density of 10^3 cells/well at 37°C under humidified atmosphere containing 5% CO_2. Triplicates of sterilized sample were taken and then incubated in serum containing media for 24 h at 37°C. 100 μL of the media from each sample was taken and transferred into each well then incubated for another 24 h. After that, MTT solution was added to each well followed by 4 hours of incubation at 37°C. Subsequently, the medium was removed and violet crystals formed were solubilized with DMSO. After shaking slowly twice for 5 seconds, the absorbance of each well was determined using Eliza reader at 570 nm. The cell viability was expressed as a percentage of the control.

2.9. Evaluation of Wound Healing Activity. The wound healing activity was evaluated by excision wound model in albino rabbits (1.7 to 2.0 kg). All *in vivo* experiments were carried out in accordance with the ethical guidelines for Animal Care and Use Committee of Medical and Pharmaceutical University (Ho Chi Minh City). The animals were anesthetized by using lidocaine 25% prior to the test. The dorsal hair of the rabbit was removed. Full thickness wound of 2.5 × 2.5 cm^2 was excised from the back of the rabbit. The wound was covered

with an equal size of drug-loaded sponge or blank sponge. The cotton gauge was used for control. The sponge was removed and replaced by fresh sponge every three days. The wound closure was observed at different periods of treatment using a digital camera. Wound area was calculated on the 0th, 3rd, 12nd, 15th and 21st days. On day 21st, histological examinations of skin wound tissue were carried out. The skin wound tissue of animal was excised, fixed with 10% formalin, and stained with hematoxylin-eosin (H&E) reagent for histological observations.

3. Results and Discussion

The composite sponges were prepared in different ratios of chitosan and gelatin (3 : 1, 1 : 1, and 1 : 3) (Figure 1). The results indicated that increasing of gelatin concentration leads to increase of folding endurance. The composite sponge SP1 showed the highest folding endurance among all composite sponges (data not shown). To examine the chemical structures of various sponges prepared under different feeding ratios, the prepared sponges were examined by FTIR spectra. The –CH– stretching vibration of the chitosan and gelatin is at 2936–2969 cm^{-1}, while peaks of chitosan at 1650 and 1560 cm^{-1} correspond to the amide carbonyl group (amide I) and the bending frequency of the amide N–H group (amide II), respectively (Figure 2). The FT-IR of composite sponge exhibited a mixture of characteristic absorptions because of amine groups of chitosan and the carboxylic acid groups of gelatin. The peaks of C = O of amide I for chitosan at 1650 cm^{-1} was shifted to 1669 cm^{-1} in composite sponges. The intensity of C = O peaks increased with the decline ratio of gelatin in composite sponge from 1 : 3 to 3 : 1 (chitosan/gelatin). These results can be attributed to the interaction of $-NH^{3+}$ of chitosan and the COO^{-} of gelatin in three composites [18, 19].

Figure 3 shows the cross-section morphology of chitosan/gelatin composite sponges. According to the figure, the sponges appeared as a very porous structure. Additionally, the roughness of chitosan/gelatin sponge surface was decreased with increasing of gelatin content (Figures 3(a)–3(c)). It was found that the large pores were observed in cross-section morphology of chitosan/gelatin sponges (Figures 3(d)–3(f)). The pore sizes were 43.06, 35.6, and 29.9 μm for SP3, SP2, and SP1, respectively. The sponges with low gelatin level represent regular pore size. On the other hand, the irregular pore size was observed in cross-section morphology of sponge with high gelatin content. The irregular pore size appearing in the sponge with high gelatin level may be deduced from the incompatibility between chitosan and gelatin molecules.

Water absorption capacity is an important parameter for biological applications and wound healing. It presents the capacity of sponge to absorb wound exudates. As the gelatin contents of prepared sponges were increased, the water adsorption capacities were significantly decreased. This is because lower water content led to smaller pore size during the preparation of the sponges. As a result, the water uptake was relatively lower. The water uptake capacity was 227%, 351%, and 2352% for ratio of chitosan/gelatin 1 : 3, 1 : 1, and 3 : 1, respectively. The high water content in prepared sponges was explained by the hydrophilic and swelling properties of gelatin [20]. The SP3 presented maximum water absorption capacity among all prepared sponges; the sponge can retain water in porous pore surrounding by cell walls. Additionally, it decreased about 10-fold for SP1 and SP2 which may attribute to the swelling and dissolving of gelatin that could decrease the pore size leading to less water absorption and therefore could not retain much water within their network structure. Furthermore, the water absorption capacity of drug-loaded sponge was not significantly different from blank sponge. The similar results were also reported in previous study [18]. These results were further confirmed by weight loss of sponges. Table 1 presents that the weight losses were 81.5, 66.5 and 16% for SP1, SP2, and SP3, respectively. The weight loss is reasonable as more gelatin can resist the dissolution in water. The water uptake may be related to the pore. The more gelatin, the smaller pores, and pore volume were observed.

The *in vitro* release profile of curcumin-sponge was conducted in phosphate buffer solution. The average percent release of curcumin was approximately 91%, 55%, and 37% for

(a) (b)

Figure 1: Prepared composite sponge with ratio of chitosan and gelatin (3 : 1) (a) curcumin-sponge and (b) blank sponge.

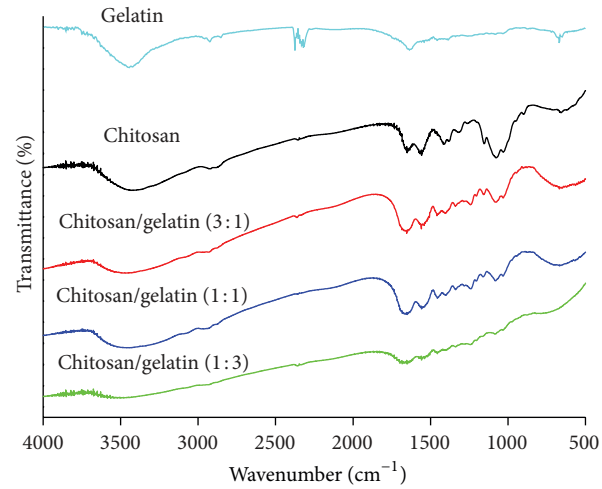


Figure 2: FT-IR spectra of prepared composite sponge with various ratios of chitosan and gelatin.

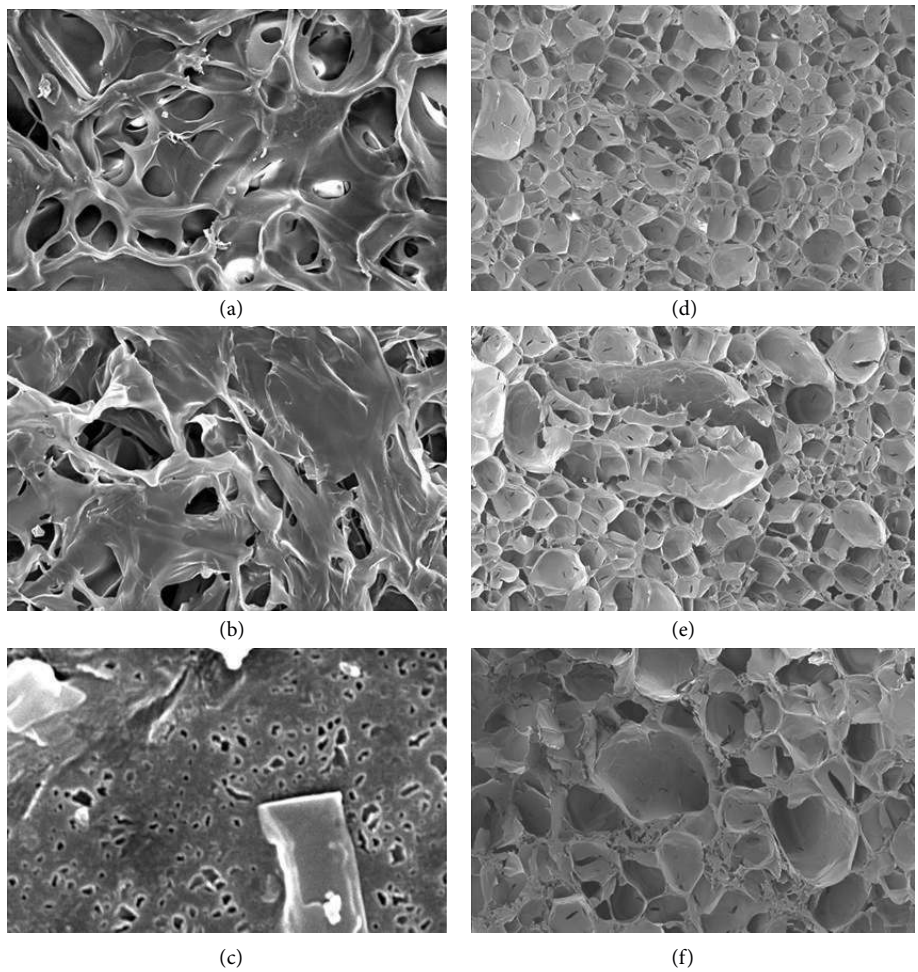


Figure 3: SEM images of surface morphology of chitosan/gelatin sponges (a) SP3, (b) SP2, and (c) SP1. Cross-section morphology of chitosan/gelatin sponges (d) SP3, (e) SP2, and (f) SP1 (scale bar at 100 μm).

Table 1: Composition and physical parameter of prepared sponges.

Sample no.	Feeding ratio		Average water uptake (%)	Weight loss (%)
	Chitosan	Gelatin		
SP1	1	3	227	81.5
SP2	1	1	351	66.5
SP3	3	1	2352	16

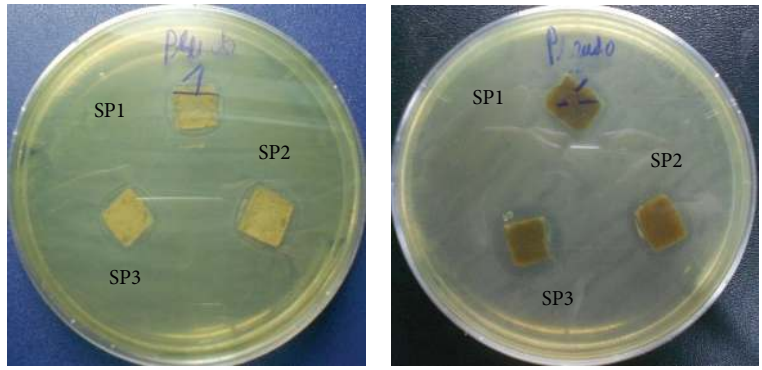
Table 2: The curcumin release profile of prepared sponges.

Time (h)	Drug release (%)		
	SP1	SP2	SP3
0	0.00	0.00	0.00
1	16.35	1.38	0.53
6	30.85	4.47	1.62
24	43.53	10.21	7.38
30	49.44	13.59	8.15
48	59.44	29.73	9.21
54	63.41	33.97	14.26
72	78.12	43.26	23.09
96	90.97	54.71	36.91

SP1, SP2, and SP3, respectively, in 96 h. The curcumin release increased with increase of gelatin content in prepared sponge (Table 2). This is because the hydrophilicity of curcumin was relatively lower. According to the partition ratio between sponge phase and water phase, the drug in the sponge having a higher gelatin content is preferred to be released, instead of being dissolved by the sponge.

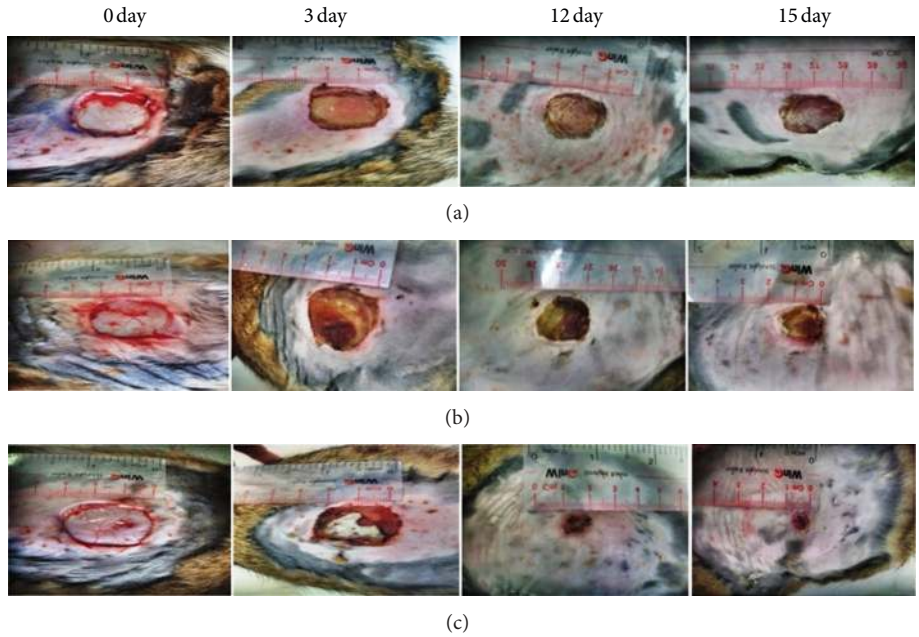
From the results of antibacterial activity studies of prepared sponges against *P. aeruginosa*, it can be seen that the inhibition zone is larger in curcumin composite sponge than that of chitosan sponge without curcumin. It can be also seen that the SP3 sponge exhibited a higher inhibition zone than those of SP1 and SP2 sponges (Figure 4). The inhibition zones were 21 mm and 17.5 mm for curcumin-loaded SP3 and SP3 without curcumin, respectively.

The cytotoxicity of prepared sponges to L929 cells was evaluated using MTT assay method. The results demonstrate the cell viability after 24 h incubation with medium released



(a) (b)

Figure 4: Antibacterial activity of prepared sponges against *Pseudomonas aeruginosa:* (a) sponges without curcumin and (b) curcumin-sponges.



(a)

(b)

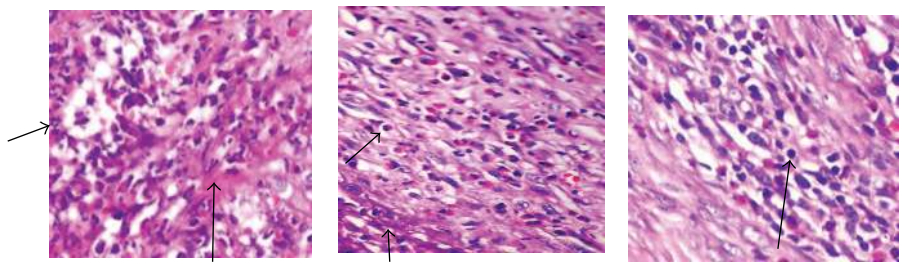
(c)

Figure 5: Photographs of macroscopic appearance of wound repair covered with (a) control, (b) blank sponge, and (c) curcumin sponge, at day 0, day 3, day 12, and day 15, respectively.

from sponges. The results present that cell viability decreased to approximately 90% in the presence of medium containing sponge without curcumin and curcumin sponge. The cell viability suggests that these sponges have generally low cytotoxicity to the L929 fibroblast cells (data not shown).

The SP3 sponge was selected for wound healing test. The percentage of wound closure in untreated and treated groups was measured on 3rd, 12nd, 15th, and 21st post wound day and the results are presented in Table 3. The wound closure was similar in three groups on 21st post wound day. The wound closure was 99.49%, 97.63%, and 94.30% for curcumin-SP3, blank SP3 and control group, respectively. It was found that the wounds treated with curcumin composite and composite without curcumin have shown faster wound healing than wound untreated group. Moreover, the wound closure on the 12nd and 15th day was significantly increased in curcumin SP3 in comparison with SP3 without curcumin (Figure 5). These results enhance the wound healing activity and could be a good material to be employed as wound dressing.

Histological Examination. The histological examination of three different kinds of wound dressing applied on skin wound of rabbit is presented in Figure 6. As can be seen in Figure 6, the tissue is in the acute stage of wound healing for control (Figure 6(a)). The tissue is under subacute stage of wound healing due to that there are many eosinophil, allergic reactions may be considered (Figure 6(b)). The



(a) (b) (c)

Figure 6: The H&E stained sections of twelve-day postsurgery of wound treated with (a) control: Short arrow—polymorphonuclear neutrophil (PMN), long arrow—fibroblast. The tissue is in the acute stage of wound healing. (b) Blank sponge (without curcumin): short arrow—eosinophil, long arrow—necrotic tissue. The tissue is under subacute stage of wound healing due to that there are many eosinophil and allergic reaction may be considered, and (c) curcumin sponge: arrow—lymphocyte, the tissue is under subacute stage of wound healing (400x).

Table 3: Wound closure of animal treated with blank sponge and curcumin sponge.

	Wound closure (%) (mean ± SD)			
Time	3rd day	12nd day	15th day	21st day
Control	15.36 ± 0.54	30.00 ± 1.05	48.80 ± 1.71	94.30 ± 1.93
SP3	14.79 ± 0.58	49.41 ± 1.93	77.96 ± 3.91	97.63 ± 4.28
Curcumin-SP3	26.53 ± 1.14	90.82 ± 2.11	95.41 ± 3.62	99.49 ± 3.81

wound is under subacute stage for curcumin-loaded sponge (Figure 6(c)). The granulation tissue and collagen alignment were more advanced in the sponge without curcurmin and curcumin-loaded sponge-treated wound as compared to the untreated wounds (control).

4. Conclusion

In this work, composite sponge of curcumin/chitosan/gelatin was prepared at the various ratios of chitosan and gelatin. The structure and morphology were characterized using FT-IR and SEM. The composite of curcumin, chitosan, and gelatin improved water uptake ability, antibacterial activity, and wound closure. Based on the results of drug release of curcumin, the higher content of gelatin in composite sponge exhibited a faster release behavior up to 240 min. These composite sponges were found to enhance the formation of collagen and wound closure *in vivo* and therefore improved the wound healing activity.

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