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Biological properties of the chitosan-gelatin sponge wound dressing ☆

Chun-Mei Deng [a], Lan-Zhen He [a], Ming Zhao [b], Dan Yang [a,*], Yi Liu [a]

[a] *Science Institute, Guangdong Ocean University, Zhanjiang 524088, PR China*
[b] *Affiliated Hospital of Guangdong Medical College, Zhanjiang 524001, PR China*

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Abstract

In order to testify the safe reliability, antibacterial property and wound healing ability in clinic application of the chitosan-gelatin sponge wound dressing (CGSWD), a lot of experiments were executed : (1) A series of toxicological evaluations of the CGSWD were performed. (2) The antibacterial properties of the CGSWD obtained by different dryness ways were investigated, and then compared the antibacterial properties with cefradine, ciprofioxacin and penicillin to ascertain how its antibacterial property is. (3) The animal wound model was made on the back of New Zealand rabbit, and treated respectively with vaseline sterile gauze, 0.2% (v/v) ethacridine and CGSWD. The wound skin tissues at the 3rd, 5th, 7th, 10th and 14th day after initial wounding were observed with naked eyes first, then all the skin tissues at the 14th day after initial wounding and normal skin without wounding were observed on SEM.

The results showed that as a kind of surgical wound dressing, the CGSWD is of safe reliability, good antibacterial property. The antibacterial effect of CGSWD dried by vacuum dryness on *Escherichia coli* K88 is better than that of penicillin, and the effect on *Streptococcus* is also better than that of cefradine. CGSWD makes the wound contract obviously, and the wound healing time of treatment with CGSWD is shorter than that with vaseline sterile gauze. Still, the scar formed after healing did not protrude over the skin surface conspicuously. The healing effect of CGSWD superior to that by 0.2% (v/v) ethacridine. Therefore, the CGSWD is a kind of excellent wound healing material.

Keywords: Chitosan; Gelatin; Wound dressing; Toxicological evaluation; Antibacterial property; Wound healing

1. Introduction

Skin is the biggest organ of human body and a barrier against environment. When it is destroyed by damage or disease, the moisture content, electrolyte and protein in wound would be lost, and the infection probability of wound increases. If the initial wound is enswathed effectively with wound dressing, the infection and dehydration of the wound could be prevented. On the other hand, that would promote wound healing, decrease complication.

Usually, the wound dressing must be preserving moisture, soft, of good permeability, non-toxicity and no pyrogen, as well as have no bad reaction and stimulation to flesh, and could promote the flesh bud growth and the skin regeneration, accelerate wound healing, decrease scars and so on.

Chitosan has many useful biological properties such as hemostasis and acesodyne activity, wound healing property, reducing scars, bacteriostasis, biocompatibility, biodegradability and so on. So, it is very suitable to be a kind of wound dressing material (HaiPeng, Yinghui, & Jianchun, 2000; Jiang, 2001; Lai, Suyuan-fen, & Liu, 1999; Masayuki, Kuniaki, & Katsuaki, 2002; Scchriest, Miao, & Niyibizi, 2000; Yu-Bey, Shu-Huei, & Fwu-Long, 2004; Zikakis, 1984).

In this study, CGSWD is made from chitosan as main material and gelatin as assistant material with some other reagents. Many former experiments have proved that CGSWD is soft, permeable and preserving moisture. CGSWD has many advantages in wound dressing. The

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* Corresponding author.
E-mail address: dana_yz@yahoo.com.cn (D. Yang).

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biological properties of CGSWD were researched to testify its safe reliability in flesh in the paper. The results showed that CGSWD has excellent biological properties.

2. Experiment

2.1. Experimental materials

2.1.1. Experimental animals

Small Kunming rats and big New Zealand rabbits, supplied from the animal experimental center in Guangdong medical college.

2.1.2. Bacteria

Escherichia coli K88, *Streptococcus*, provided by microorganism office in Guangdong Ocean University. LB liquid medium and LB solid medium: prepared in conventional way.

2.1.3. Medicine-sensing test paper

Cefradine (30 μg), ciprofioxacin (5 μg) and penicillin (10 μg), purchased from the clinical laboratory in the Central Hospital of Guangdong Agricultural Cultivation.

2.1.4. Pharmic materials

0.2%(v/v) ethacridine, vaseline, aseptic sterile gauze, purchased from market.

2.2. Apparatuses

XL30-EDAX SEM (Philips, Holand), XLD-0.1J Electron tension tester (No. 2 experimental factory in Changchun, China), Daojing UV/VIS-160A spectrophotometer.

2.3. Experimental methods

2.3.1. Preparation of CGSWD (He, Liu, & Yang, 2006)

Mixing chitosan and gelatin into solution, adding appropriate cross-linking agent and some other assistants, then freezing and shaping it under low temperature suddenly. When it solidified, dehydrating it. Then the CGSWD (1.5 mm thickness) with unique porous membrane structure has been made.

2.3.2. Animal toxicological evaluation (American society of Testing Material, 1990; Yu, Zheng, Deng, Luo, & Sun, 2005)

Animal toxicological evaluation was taken according to the national sanitation ministry criterion WS5-1-87, the international criterion <ISO> and the American criterion <ASTM>.

2.3.3. Preparation of bacterium suspending liquid

Inoculating *E. coli* K88 and *Streptococcus* on LB liquid state medium, culturing them at 37 °C for 24 h, then picking the single cultured bacterium onto 3 ml LB liquid state with concussion at a rate of 200 r/m and at 37 °C.

2.3.4. Comparison of antibacterial properties of CGSWD

Under an asepsis operation condition, the bacterium suspending liquid at a does of 100 μl were evenly spread on LB solid state medium, then putting the CGSWD (7 mm in diameter) on that medium after 30 min. Meanwhile, so did the bacteriophage medicine-sensing test papers for comparing their antibacterial properties. Let all samples culture at 37 °C for 24 h. Finally the antibacterial circles were observed.

2.3.5. Preparation of animal wound models

Six New Zealand rabbits with a body weight range from 2.25 to 2.50 kg were used in this study, half male and half female. Six wounds (0.3 cm thickness from coat to muscle, 2 cm diameter) were made on the back of rabbits at the distance of 2 cm from the vertebral column, which were arranged symmetrically. Lastly, all animal wound models were washed with physiological brine for using.

2.3.6. Treatment of animal wounds

Dividing all animal wound models on the back of New Zealand rabbits into three groups. The first group of that were treated with 0.2% (v/v) ethacridine at a does of 0.2 ml, the second group coated with CGSWD, the third group treated with nothing coated with vaseline aseptic sterile gauze. Every wound was banded up with asepsis gauze and renewed medicines every other day as well as observed.

2.3.7. Animal wound measuring index

The wounds were covered with transparent membranes instantly after wounding, and at the 3rd, 5th, 7th, 10th, 14th day after the initial wounding, then the scope of wounds was described with color pen. Cutting the membranes as the scope of wounds and weighing them, then changing them into areas. All the data are the mean from standard calculation.

2.3.8. Evaluation method

The pellicle and the flesh bud at the 3rd, 5th, 7th, 10th, 14th day after medicine used were observed in order to study its growth.

2.3.9. SEM observation of animal wound tissues

Taking the new skin tissues at the 14th day after initial wounding and normal skin without wounding as SEM samples. Fasting the samples with glutaraldehyde, than observing them on SEM.

3. Results

3.1. Toxicological evaluations of the CGSWD

Animal toxicological evaluation was taken according to the national sanitation ministry criterion WS5-1-87, the international criterion <ISO> and the American criterion <ASTM>.

3.1.1. Acute and toxic experiment on the whole body

Every animal is observed in succession at 4, 24, 48 and 72 h after being injected. The animal fettle of experimental groups was quite well. There was no toxic symptom occurred in both experimental group and control group. No animal died after infection for 72 h. The average weight of animals at 4, 24, 48 and 72 h after being injected was recorded respectively in Table 1.

3.1.2. Pyrogen experiment

Table 2 showed the body temperature change of big rabbits after being injected leaching solution. The result indicated that the temperature of every rabbit averagely rose below 0.5 °C, and the whole increase of animal temperature of three rabbits is only 0.9 °C. That is lower than the criterion of 1.6 °C prescribed in 'Pharmacopoeia of the People's Republic of China', which denoted that CGSWD is suitable with the prescription of pyrogen examination. There is no pyrogen reaction taken place on the rabbits after being injected.

3.1.3. Primary skin irritation experiment

Table 3 is the result of skin irritation experiment. The result showed that CGSWD has no irritation on skin, which effect is the same as the negative physiological brine control group.

3.1.4. Intracutaneous injection experiment

Table 4 is the result of intracutaneous injection experiment. From Table 4, it could be seen that the animals have no erythema and no dropsy after being injected leaching solution of CGSWD by plant oil and physiological brine separately for 24, 48 and 72 h. There is no difference compared with the physiological brine control groups and plant oil control groups, which denotes that the injected leaching solution of CGSWD has no skin irritation.

3.1.5. Eye conjunctiva irritation experiment

The results of the eye conjunctiva irritation experiment is that: There is no any stimulation occurred when the leaching solution of CGSWD by physiological brine continuously contacts the eyes during 14 days, and no inflammation cell detected in ball conjunctiva pellicle cells by checking smear inspection. The experimental results are negative, which shows that CGSWD belongs to non-irritant material.

Table 3
The result of skin irritation experiment

Time	Negative control group	Experimental group	Positive control group
1 h	No erythema, no dropsy	No erythema, no dropsy	Erythema, dropsy
24 h	No erythema, no dropsy	No erythema, no dropsy	Erythema, slight dropsy
48 h	No erythema, no dropsy	No erythema, no dropsy	No erythema, no dropsy

3.1.6. Hemolysis experiment

Table 5 is the absorbency values* in hemolysis experiment.

In the formula, As is for the absorbancy of samples, An for the absorbancy of the negative control group, Ap for the absorbancy of positive control group.

Hemolysis rate of 1.23% is lesser than the criterion of 5%, which shows that CGSWD does not have hemolysis function. Remarkably, the samples must be prepared according to the requirement. If the sample is too thick, the total area that sample contact solvent certainly increase and the solvent would immerge into the inner structure of sample, then the hemolysis rate measured becomes higher.

3.2. Antibacterial properties of CGSWD

3.2.1. Antibacterial properties of CGSWD by natural dryness and vacuum dryness

CGSWD were naturally dried at room temperature, and dried under vacuum condition at 45 °C and at 1 MPa for a certain time. Fig. 1 presents its antibacterial properties to *E. coli* K88 and *Streptococcus* respectively.

Table 1
The average weight of animals in experimental group and control group

Number	Experimental group1#(g)	Experimental group2#(g)	Control group1#(g)	Control group2#(g)
Initial weight	20.44	18.92	19.57	21.42
Weight after infection for 24 h	21.73	20.58	20.33	19.18
Weight after infection for 48 h	20.38	18.28	19.08	20.43
Weight after infection for 72 h	21.62	19.48	18.13	19.73

Table 2
The temperature change of the rabbits after being injected leaching solution

Number of rabbits	Gender	Weight (kg)	Basic temperature (°C)	Temperature after injecting for 60 min (°C)	Temperature after injecting for 120 min (°C)	Temperature after injecting for 180 min (°C)
1#	Male	24.83	38.6	38.9	38.7	38.7
2#	Male	24.77	38.4	38.6	38.3	38.8
3#	Male	26.02	38.7	38.9	38.8	39.0

Table 4
The result of intracutaneous injection experiment

Time	Physiological brine control group	Leaching solution of CGSWD by physiological brine group	Plant oil control group	Leaching solution by plant oil group
24 h	No erythema, no dropsy	No erythema, no dropsy	No erythema, no dropsy	No erythema, no dropsy
48 h	No erythema, no dropsy	No erythema, no dropsy	No erythema, no dropsy	No erythema, no dropsy
72 h	No erythema, no dropsy	No erythema, no dropsy	No erythema, no dropsy	No erythema, no dropsy

Table 5
The absorbency values in hemolysis experiment

Experiment groups	Physiological brine control group	Distilled water control group	Leaching solution of CGSWD by physiological brine group
Absorbancy (%)[a]	0.021	0.487	0.027

[a] Absorbency is average value. Hemolysis rate $= \frac{(As-An)}{(Ap-An)} \times 100\% = 1.23\%$.

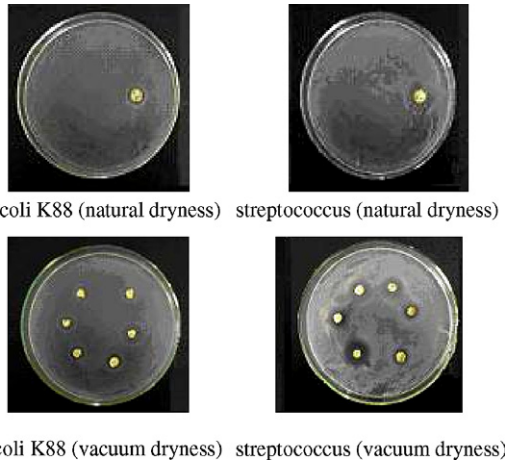

Fig. 1. Comparison of antibacterial properties of CGSWD after vacuum dryness and natural dryness.

Tables 6 and 7 are the sizes of antibacterial circle of CGSWD by natural dryness and vacuum dryness.

These results indicated that CGSWD could play antibacterial role to the growth of two kinds of bacteria in a certain degree. CGSWD by natural dryness has antibacterial effect on *E. coli* K88 and *Streptococcus*, and the antibacterial effect on *Streptococcus* is superior to that on *E. coli* K88. In addition, the antibacterial effect of CGSWD by vacuum dryness on *Streptococcus* is better than that on *E. coli* K88. Meanwhile, its antibacterial property has

Table 6
Sizes of antibacterial circle of CGSWD by natural dryness

Bacterium	Diameter of antibacterial circle (mm)[a]
K88 *E. coli* K88	12
Streptococcus	14

[a] Data in table are mean of every antibacterial circle diameter.

Table 7
Sizes of antibacterial circle of CGSWD by vacuum dryness

Bacterium vacuum dryness time	Diameter of antibacterial circle (mm)[a]					
	5 min	10 min	15 min	20 min	25 min	30 min
E. coli K88	11	17	13	13	13	12
Streptococcus	13	23	19	16	16	14

[a] Data in table are mean of every antibacterial circle diameter.

certain rule along with the vacuum dryness time prolonging. Its antibacterial effect on two kinds of bacteria would become the best when the vacuum dryness time is 10 min. In a word, the antibacterial properties of CGSWD obtained after vacuum dryness is superior to that after natural dryness no matter whether it is *E. coli* K88 or *Streptococcus*. That is because the vacuum dryness has more positive influence than natural dryness on the disconnection of hydrogen bonds.

3.2.2. Comparing the antibacterial properties between CGSWD and antibiotics

Fig. 2 presents the antibacterial properties of antibiotics to *E. coli* K88 nd *Streptococcus*, including ciprofioxacin, cefradine and penicillin.

Table 8 is the comparison of antibacterial properties to *E. coli* K88 and *Streptococcus* between CGSWD and anti-

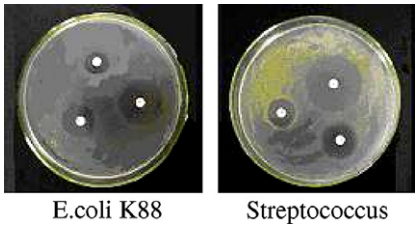

Fig. 2. Comparison of antibacterial properties of antibiotics.

Table 8
Comparison of antibacterial properties between CGSWD and antibiotics

Bacteria time	Antibacterial diameter (mm)[a]			
	Vacuum dryness for 10 min	Ciprofioxacin	Cefradine	Penicillin
E. coli K88	17	29	24	12
Streptococcus	23	29	22	46

[a] Data are mean of every antibacterial circle diameter in table.

biotics including ciprofioxacin, cefradine and penicillin. Comparing with several kinds of common antibiotics, CGSWD by vacuum dryness for 10 min has better antibacterial effect on *E. coli* K88 than that of penicillin, but lesser than that of ciprofioxacin and cefradine; its antibacterial properties to *Streptococcus* by vacuum dryness for 10 min are better than that of cefradine, but lesser than that of ciprofioxacin and penicillin.

3.3. Effect on promoting the wound healing

3.3.1. Visual observation of wound

The wounds treated with different ways have obvious difference in promoting the epidermis growth and the wound shrinkage. At the 3rd day after initial wounding, there was granulation tissue obviously grown in experimental group and medicine group samples, lamellas or dot epidermis occurred partially. The skin of the wound edge contracted evidently. The wound condition in blank group was inferior to that in other groups, and the wound condition in experimental group is superior to that in medicine group and in blank group.

At the 7th day after initial wounding, all the wounds in experimental group and in medicine group were not infected, and many granulation tissues grew under epidermis and in flesh, which could mend wounds. However, there were eight twelfths wounds was wet in the blank group, and exudates still existed. The wound shrinkage was inconspicuous. Only lamella granulation tissue could be seen. There was not obvious fresh epidermis. At the 14th day after initial wound, all of scabs cicatrized. In experimental group and medicine group, the scab skins naturally fell off during a week. In blank group, three twelfths wounds were infected. The rest of wounds cicatrized, and the scab skin naturally fell off during a week. The experimental result is in Table 9. After each group of wound scab skin fell off naturally, the scars of each group of wound formed had evident difference. In the medicine group, the scars projected over the skin surface more obviously. In the blank group, scars formed are evidently rugged, and most of them were concave below the skin surface. But in experimental group, the scar projected over the skin surface inconspicuously, the wound restoration was preferable.

Fig. 3 is SEM ultra-micrographs of histological sections of both the new wound skin treated with different ways and the skin without wounding at the 14th day after initial wounding.

From SEM ultra-micrographs, it could be seen that the new skin tissues of the wound treated with various ways are obviously different. The normal skin tissue without wounding has naturally arranging collagen fasciculi (J0). There are even interspaces among the collagen fasciculi. The collagen fasciculi of the new skin tissue (J1) treated with 0.2% (v/v) ethacridine are thin, dense and tightly arranged, and few interspaces exists. The new skin in blank group (J2) is obviously thinner than the skin without wounding. The collagen fibrous bundle is scarce, and the interspaces are big and uneven. However, the collagen fasciculi of the

Table 9
Comparing of the wound area among medicine group, blank group and experimental group (cm^2)

Samples	Blank group	Medicine group	Experimental group
Instanter	2.3 ± 0.3	2.3 ± 0.3	2.3 ± 0.3
3 d	2.0 ± 0.3	1.7 ± 0.3	1.8 ± 0.3
7 d	1.1 ± 0.3	0.5 ± 0.3	0.8 ± 0.3
14 d	Scabs basically cicatrized. Three twelfths wounds were infected. The scab skins naturally fell off, and scars formed were evidently concave below the skin surface	All of scabs cicatrized. The scab skins naturally fell off, the scars projected over the skin surface evidently	All of scabs cicatrized. The scab skins naturally fell off, the scars projected over the skin surface inconspicuously

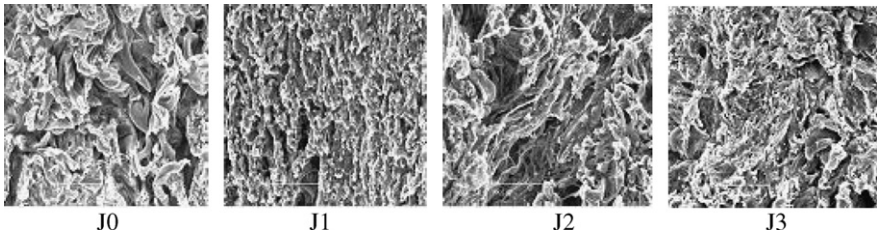

Fig. 3. SEM ultra-micrographs of every group at the 14th day after initial wounding (in the figure, J0 is the back skin tissue without wounding, J1 is the skin tissue in medicine group; J2 is the skin tissue in blank group; J3 is the skin tissue in experimental group).

new skin tissue (J3) treated with CGSWD is arranged more naturally. The interspaces among collagen fasciculi are more even, and the thickness of its new skin is the same as that of the skin without wounding.

4. Discussion

4.1. Toxicological evaluations of CGSWD

A series of toxicological evaluations of CGSWD were performed. All of evaluation results conform to the demands in relative criterion. That proves CGSWD is safe reliability as a surgical wound dressing.

4.2. Evaluations of antibacterial properties of CGSWD

Many research denoted that the antibacterial properties of chitosan are main origin in the replacing radical amido ($-NH_2$) with positive charge in its molecule chains. The number of dissociating amido has influence over the antibacterial properties of chitosan (Hou & Gu, 2001; Lu, 2001; Zheng, Wang, & Zhong, 2005) which has been testified in our study. When CGSWD are dried, some hydrogen bonds are disconnected between molecules of chitosan and water, gelatin and water, chitosan and gelatin. So, amidoes ($-NH_2$) are exposed. The antibacterial properties of CGSWD accordingly improve. Simultaneously, the formation of amido bonds leads to decrease the number of dissociating amidoes ($-NH_2$), reduces antibacterial properties of CGSWD. Hence, there exists a critical point in the course of both of disconnection of hydrogen bonds and formation of amido bonds, at which the antibacterial property of CGSWD is best. At the beginning of dryness, the number of hydrogen bonds decrease along with the water volatilization that the amidoes ($-NH_2$) are exposed. The number of amidoes ($-NH_2$) is more than that before dryness. The antibacterial property of CGSWD improves as the dryness time prolongs. After a period of time, along with dryness degree increasing, the amidoes ($-NH_2$) would form amido bonds when combined with carboxyl groups (–COOH) in chitosan, which leads to decrease the number of amidoes ($-NH_2$), then the antibacterial property of CGSWD gradually decreases. Still, the antibacterial property of CGSWD is related to bacteria structure. In bacteria, 50–80% solid components are protein. The protein consists of facultative amino acid. p*I* of gram positive bacteria is 2–3, and p*I* of gram negative bacteria is 4–5. So all the bacteria comprise negative charge under approximately neutral circumstance, especially the gram masculine bacteria (Chen, Dong, & Zhang, 1994) that it is easy to combine with CGSWD which are positive ion protonated. Then the electriferous state of protein in bacteria is transformed, the physiological function of the bacteria is disturbed, it is difficulty for bacteria to reproduce and the bacterial activity is finally restrained. *E. coli* K88 belongs to gram-negative bacterium, and *Streptococcus* belongs to gram-positive bacterium. Therefore CGSWD has good antibacterial effect on *E. coli* K88 and *Streptococcus* in the course of experiment, which the effect on *Streptococcus* is superior to that on *E. coli* K88.

4.3. Biological properties of CGSWD

In general, chitosan could induce hyperplasia of partial macrophage and build up its activity. The mechanism of increasing macrophages is that (Chen, Dou, & Luo, 2005; Li, Chen, & Wang, 2002): (1) Chitosan is a positive chemoattractant of macrophages. It could abstract monocyte from blood vessel, and then monocyte get together into macrophage in tissue; (2) Chitosan could stimulate directly cells in partial tissue reproducing, and then the cells change into macrophages. The hyperplasia and activity improvement of macrophages could make for the wound healing and anti-infection.

Moreover, gelatin is made from animal collagen after being breaked blandly. Its main components are collagen, and a few protein amylases and certain organic substances. Collagen owns so strong bioactivity that it could take part in the movement, differentiation and multiplication of cell. Meanwhile, it also make bone, tendon, cartilage and skin have certain mechanical strength. The new skin in blank group is obviously thinner than the new skin without wounding, and the collagen fasciculi are scarce, the interspaces are big and uneven. This denotes that the cell reproduction is not good, and the wound restoration is bad. There is hyperplastic collagen cells existed in the new skin in ethacridine medicine group, which makes the new skin thick. At the 14th day after initial wounding, all of scabs cicatrize with no infection. CGSWD has the same pharmacological function as 0.2% (v/v) ethacridine. All of the scabs also cicatrize. In addition, the new skin growth treated with the CGSWD is superior to that treated with ethacridine.

5. Conclusion

CGSWD is safe reliability as a surgical wound dressing. It provides with excellent antibacterial properties. The antibacterial effect of CGSWD by vacuum dryness on *E. coli* K88 is better than penicillin, and it has better antibacterial effect on *Streptococcus* than cefradine too. The effect of CGSWD on wound healing is evident. Its wound healing speed is quicker than vaseline sterile gauze; the formed scar is not bulge distinctly. This is better than that treated with 0.2% (v/v) ethacridine. Hence, CGSWD is excellent wound healing material.

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