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Chitosan coated cotton fiber: preparation and physical properties

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Abstract

A new cotton fiber with a chitosan coating (CCCF) was prepared by the oxidation of a cotton thread with potassium periodate at 60°C in water and subsequent treatment with a solution of chitosan in aqueous acetic acid. Infrared spectra of the CCCF suggested the formation of Schiff's base between the chitosan and the oxidized cellulose. Kjeldahl nitrogen analysis of the CCCF showed that the maximum percentage of chitosan introduced into the cotton fiber was 1.58% (w/w). Treatment of the fiber with 2′,7′-difluoro fluorescein (an amino group-specific probe) followed by fluorescent microscopic analysis revealed that the modification with chitosan occurred on the surface of the cotton fiber. Scanning electron microscopy (SEM) photographs showed that the surface of the CCCF was slightly changed after the series reaction. However, the mechanical strength of the cotton thread, which was oxidized by the potassium periodide solution at a concentration of less than 2.0 mg/ml, was found to be almost the same as the original cotton thread. Furthermore, a model experiment for the controlled release of the drug was preformed using shikonin, a component of a Chinese medicine, suggested potential usefulness of the CCCF as a supporter for the controlled release of drugs.

Keywords: Cellulose; Chitosan; Potassium periodide oxidation; Cotton fiber; Controlled release; Shikonin

1. Introduction

Chitin, a major component of the shell of crab and shrimp, is one of the most abundant natural polysaccharides with a large unexplored commercial potential. Chitosan is partially or completely *N*-deacetylated chitin, and mainly consists of β-(1,4)-linked 2-amino-2-deoxy-β-D-glucopyranose. In recent years, a number of investigations have been carried out to exploit the potential applicability of chitosan (Adachi, Kobayashi & Takahashi, 1987; Hirano, Yamaguchi & Fukui, 1990; Shinonaga, Kawamura & Yamane, 1992; Tokura et al., 1987). Since chitosan has unique physiological and biological properties, it is regarded as a versatile starting material for the preparation of various biomedical products (Kulpinski, Nishimura & Tokura, 1997; Nishimura, 1997; Yagi et al., 1998).

Cellulose, which has been known to have good physical properties, has been widely used as construction material, paper, and clothes. During the course of our studies on the synthesis of new cellulose-based materials (Ogawa, Sato, Miura, Tokura & Takai, 1991; Sakairi, Asano, Ogawa, Nishi & Tokura, 1998; Shirai, Sakairi, Nishi & Tokura, 1997), we examined the preparation of a cellulose–chitosan complex. Although similar cellulose derivatives have been recently reported, the methods for the synthesis have been limited to a blended chitosan particle on the cellulose fiber (Seo, 1993), treatment of the cellulose fiber with cross-linking agents (Shin & Holme, 1994) or irradiation of the cellulose by ultraviolet light (Shin, Tokino, Ueda & Suzuki, 1998). We would like to now report a novel method for the modification by oxidizing the cellulose fiber with potassium periodate and subsequent Schiff's base formation.

2. Experimental

2.1. Materials

All chemicals used for the following investigations were of analytical grade. Chitosan with an *N*-deacetylation degree of 0.80 ($\eta = 0.01675P$) and cotton spinning thread were obtained from Kyowa Techos Co., Ltd. (Chiba, Japan) and Fuji Spinning Co., Ltd. (Osaka, Japan), respectively. Shikonin was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) and used without further purification.

2.2. Oxidation of cotton thread with potassium periodate

A reel of cotton spinning thread (0.3 g) around a stainless bobbin, which had many holes, was immersed in solutions

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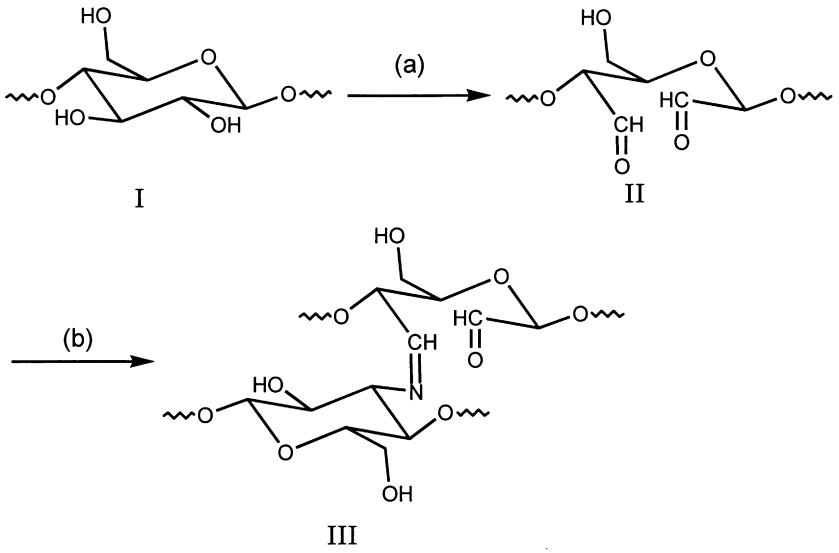

Scheme 1.

of potassium periodate in deionized water (400 ml) ranging in concentrations from 0.1 to 5.0 mg/ml. The solution was then stirred for 1 h at 60°C. The cotton thread was washed with deionized water several time to remove the oxidant, and soaked in deionized water (400 ml) with stirring for 24 h at ambient temperature. This oxidized thread was used for the next reaction without drying.

2.3. Coated by chitosan

A chitosan solution was prepared by stirring a dispersion of chitosan (8.0 g) in 2% (v/v) aqueous acetic acid solution (400 ml) for 1 h at 60°C. The above mentioned reel of oxidized cotton thread was immersed in the chitosan solution with constant stirring for 2 h at 60°C, washed with deionized water several times, and soaked in deionized water (400 ml) with stirring for 24 h at ambient temperature. The resulting cotton thread was dried at 60°C under vacuum for 6 h to produce the modified cotton thread.

2.4. Measurement

2.4.1. Chitosan content of CCCF

Chemically modified cotton thread samples were precisely weighed and then suspended in concentrated sulfuric acid (5 ml). Five drops of hydrogen peroxide (30%) was added to the suspension and the mixture was heated under reflux until the solution became transparent and colorless. The resulting solutions were subjected to Kjeldahl nitrogen analysis. The chitosan content in the CCCF was calculated from the nitrogen percentage on the basis of the calibration curve for the weight of chitosan and titration value.

2.4.2. Instrumental analysis

The CCCF sample, which contained 1.58% chitosan, was used for the following instrumental analyses. Infrared spectra were recorded using a Horiba FT-210 spectrophotometer with a potassium bromide pellet. Scanning electron microscopy (SEM) photographs were taken on a Hitachi S-2400 instrument operating at 12–18 kV after sputtering with gold. The tensile strength and elongation of the cotton thread were measured with a Shimadzu AGS-500D Auto graph tension tester. The length of the sample thread was 25 mm, the rate of extension was 2 mm/min, and the test was performed at room temperature.

2.5. CCCF load and release shikonin

A dispersion of shikonin (2.0 g) in deionized water (1.0 l) with stirring for 30 min at 80°C produced a shikonin solution. The modified cotton thread (2.7 g) was added to the shikonin solution (500 ml) and stirred for 12 h at the same temperature, and then the colored thread was vigorously washed with a small amount of deionized water. Unmodified cotton thread (2.7 g) was also treated using the same procedure to produce the control sample. The shikonin-treated thread and the control sample were separately added to an isotonic sodium chloride solution (100 ml) in two Erlenmeyer flasks. Each flask was shaken for 24 h at 37°C. The shikonin released into the sodium chloride solution was measured with a Hitachi F-4500 fluorescence spectrophotometer; the excitation wavelength was 262 nm and the observations were made at 465 nm. The solutions were replaced with fresh sodium chloride solutions every 24 h. The time-course release of shikonin was calculated from the fluorescent intensity at 465 nm.

2.6. Confirmation of chitosan by fluorescence microscopy

A small amount of modified cotton thread (25 mm) and unmodified cotton thread (25 mm) were immersed in a

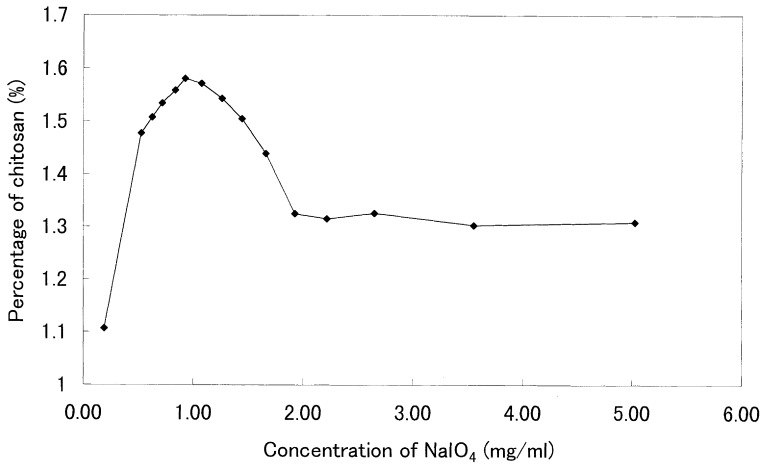

Fig. 1. Relationship between the amount of chitosan introduced into the cotton fiber and the concentration of $NaIO_4$ used for the oxidation.

solution of 2′,7′-difluoro fluorescein in 0.05 M phosphate buffer at pH 6.5 for 24 h at room temperature. The resulting threads were then sufficiently washed with 0.05 M phosphate buffer at pH 9.0. The resulting samples were examined under an Olympus IX-FLA fluorescence microscope with a magnification of 600.

3. Results and discussion

3.1. Preparation of CCCF

The preparation process of CCCF is summarized in Scheme 1 (reagents and conditions: (a) $NaIO_4$, 60°C, 1 h;

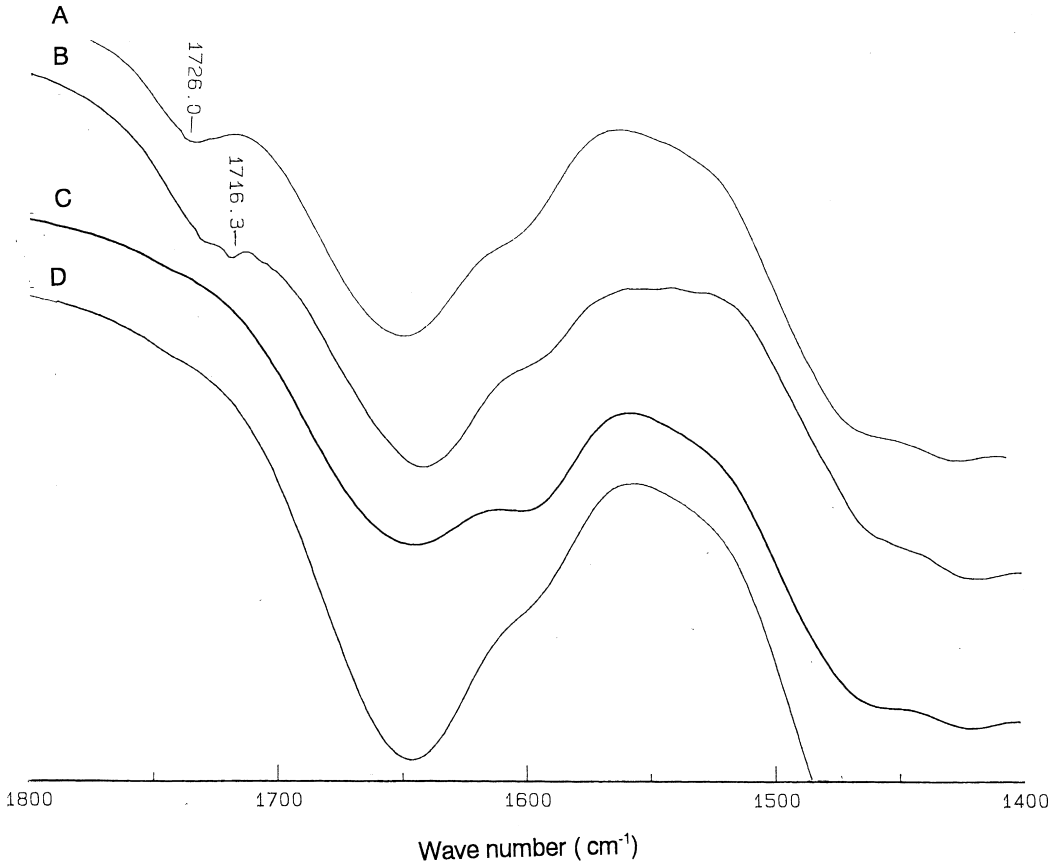

Fig. 2. Infrared spectra of: (A) cellulose fiber oxidized with $NaIO_4$; (B) chitosan coated cellulose fiber (CCCF); (C) CCCF reduced with $NaBH_4$; and (D) original cellulose fiber.

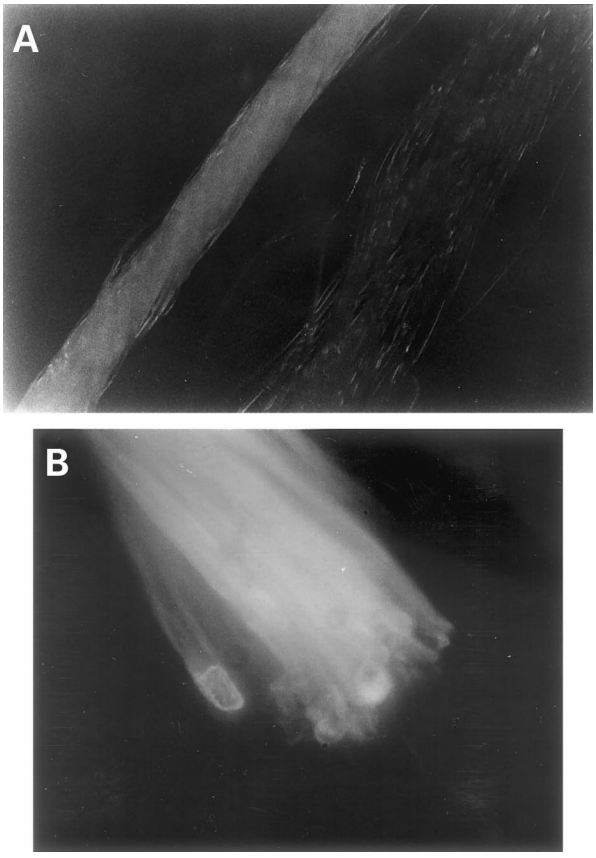

Fig. 3. Fluorescence microscopy of original cotton fiber (A right) and CCCF (A left), and cross-section of CCCF (B).

(b) chitosan, CH_3COOH (2% (v/v)), 60°C, 2 h). According to the reported procedure (Jackson & Hudson, 1937), the cotton thread was first oxidized by potassium periodate to cleave the 2,3-vicinal diol of the cellulose glucose units (I), giving the so-called dialdehyde cellulose (II). The resulting aldehyde group on the cellulose fiber would possess the ability to couple with an amino group of chitosan. Upon treatment of the thread with a chitosan solution in acetic acid, the formation of a Schiff's base went smoothly to give CCCF (III). The amount of chitosan introduced into the CCCF was determined by the Kjeldahl nitrogen analysis. Fig. 1 shows the relationship between the concentrations of potassium periodate used for the oxidation of the cellulose fiber and the amount of chitosan introduced into this novel fiber.

The amount of incorporated chitosan increased with the oxidant concentration during the initial stage, whereas it decreased at higher concentrations of potassium periodate. The maximum fixed chitosan was 1.58% of the weight of threads. When the oxidant concentration was over 2.0 mg/ml, the chitosan content became nearly constant at 1.3%. These phenomena are explained by considering the difference in the reaction site of the oxidation and Schiff's base formation in the cotton fiber. During the oxidation, the small periodate ion might be able to enter the cellulose fiber interior and the glucose unit both inside and on the surface of the cellulose fiber may be oxidized. On the other hand, chitosan is a huge molecule that cannot enter the fiber, and the modification with chitosan occurred only on the surface of the cellulose fiber. Fluorescent microscopic analysis shown in Fig. 3 supported these speculations.

The reaction process was monitored by infrared spectroscopy. Fig. 2 shows the spectra of cellulose, cellulose oxidized with sodium periodate, CCCF and CCCF reduced with sodium borohydride. The characteristic absorption band of the oxidized cellulose clearly appeared at $1726\ cm^{-1}$ due to the stretching vibration of the C=O double bond of the aldehyde group. After treatment with chitosan, the characteristic absorption band shifted to $1716\ cm^{-1}$ suggesting that the Schiff's base (C=N double bond) was formed between the aldehyde group and chitosan. Furthermore, the absorption at $1716\ cm^{-1}$ disappeared by reduction with $NaBH_4$. The infrared spectroscopic analysis results suggested that the formation of the Schiff's base occurred between the oxidized cellulose and chitosan.

3.2. Fluorescence microscope of CCCF

We next examined the fluorescence microscopic analysis using 2′,7′-difluoro fluorescein, an amino group specific probe that has been widely used in biochemistry. Thus, CCCF was treated with the fluorescent dye in phosphate buffer at pH 6.5 and then subjected to fluorescence microscopic analysis. As shown in Fig. 3A, the resulting CCCF fluoresces a light green color, whereas the original cotton fiber had almost no fluorescence. A fluorescent microscopic photograph of a cross-section of the fiber is shown in Fig. 3B, which showed a bright line on the border the fiber. These results suggest that chitosan was introduced on the surface of cotton fiber by a series of reactions.

3.3. Scanning electron microscopic analysis

The surfaces of CCCF and its synthetic intermediates were morphologically observed by SEM. Many small grains were observed on the surface of the CCCF as shown in Fig. 4B. On the other hand, the surface of the oxidized cotton fiber had many long and narrow lines (Fig. 4A). From the SEM analysis, it was clear that the CCCF surface had a unique morphological form, which is different from the unmodified cotton fiber that showed a smooth surface.

3.4. Tensile property of the modified thread

The oxidation by potassium periodate breaks to some extent the crystalline structure of cellulose in the original cotton fiber, therefore, it may weaken the tensile property. Fig. 5 shows the relationship of the breaking tensile strength and elongation of the modified thread versus oxidant concentration.

As we expected, the breaking tensile strength and elongation of the modified thread did not produce remarkable changes in the oxidation for the oxidant concentration

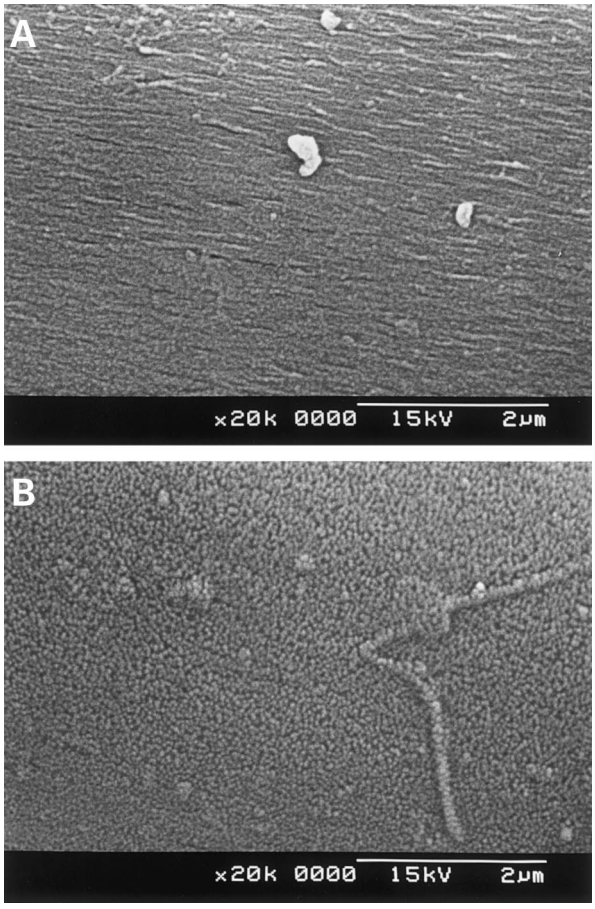

Fig. 4. SEM photomicrographs of: (A) oxidized cotton fiber; and (B) chitosan coated cotton fiber.

range of 0.0–2.0 mg/ml. However, both decreased when the oxidant concentration was over 2.5 mg/ml, probably due to breaking down the cellulose crystallization.

3.5. The loading and release of shikonin

Shikonin is extracted from the purple root of a Chinese crude drug, shikon (*Lithospermum officinale*). Shikonin is well known to have antibacterial, anti-inflammatory, anti-tumor, and wound healing activities (Ozaki, Sakaguchi & Tujimura, 1998; Sekine, Kojima & Matsumoto, 1998; Sekiya, Kadota & Katayama, 1997). The chemical structure of shikonin is shown in Fig. 6. The loading and controlled release of shikonin using our newly synthesized cellulose fiber would be applicable to making underwear for atopic dermatitis. As mentioned above, CCCF has a polycationic chitosan residue, which may interact with shikonin that has an acidic function. After treatment of the CCCF and the original cotton thread with a solution of shikonin at 80°C, release of shikonin into an isotonic aqueous sodium chloride was examined for 8 days. These results are summarized in Table 1, which showed that a larger amount of shikonin was released from CCCF.

Table 1
Release of shikonin adsorbed on the cotton threads

Time (days)	4	6	8	Remaining[a]
Fluorescent intensity[b] (CCCF)	6.8	6.2	5.0	77.4
Fluorescent intensity[b] (cotton thread)	4.4	3.7	3.4	29.4

[a] Remaining shikonin on the threads were extracted with ethanol and measured in the same conditions.
[b] Excitation wavelength was 262 nm and the observations were made at 465 nm.

After the 8-day experiment, the remaining shikonin on the threads was extracted with ethanol and subjected to fluorescent analysis. These results suggested that 2.3 times as much shikonin was adsorbed on CCCF and then slowly released.

4. Conclusions

In conclusion, the chemical modified cotton thread with a chitosan coating was obtained by the reaction between aqueous chitosan acetic acid and the oxidized cotton fiber. It was demonstrated that the chitosan molecule through the

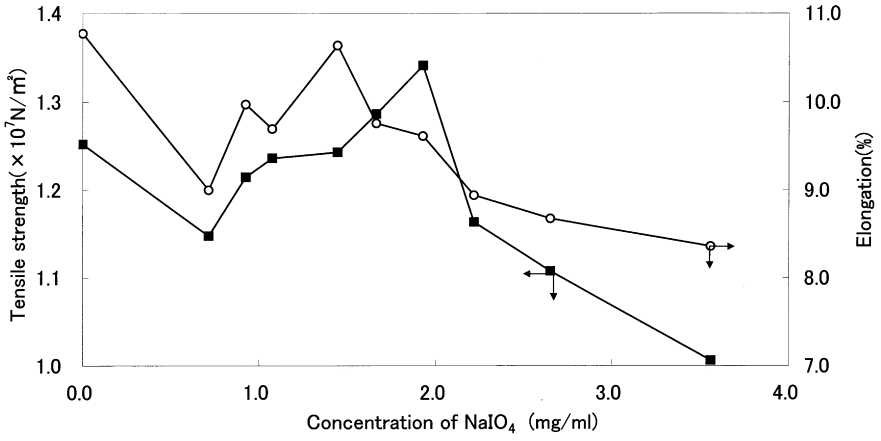

Fig. 5. Change in the breaking tensile strength (■) and elongation to break (○) of modified threads against concentration of $NaIO_4$ used for the oxidation.

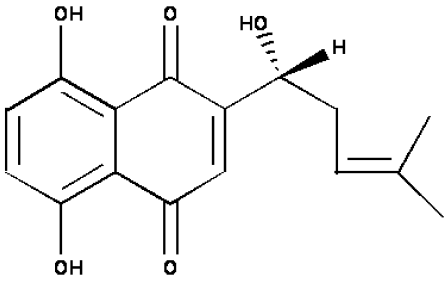

Fig. 6. Chemical structure of shikonin.

C=N double bond combined on the cotton fiber surface and oxidation did not have a significant effect on the tensile property.

With the chitosan coating, the cotton fiber surface became physiologically and biologically active. Since the chemical reaction activity of the amino group is greater than the hydroxyl group of cellulose, the fiber has more potential for still more chemical modification. From the SEM analysis, the smooth surface of the cotton fiber became coarse, which suggested that the ability of drug adsorption becomes greater. We have tried the control release of the herb medicine shikonin and obtained a good result such that this novel thread is suitable as a sustained release drug carrier.

Acknowledgements

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