



Original article

Does Sericin, as a Novel Pleurodesis Agent, Have Higher Effectiveness Compared to Talcum Powder, Doxycycline, and Silver Nitrate Pleurodesis? ☆

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ABSTRACT

Introduction: The usefulness of sericin as pleurodesis agent has previously been described. Present study aims to compare sericin pleurodesis regarding success, effectiveness, tolerability, and side-effects.

Methods: Adult, 12-week-old Wistar-albino rats ($n=60$), divided to five groups as sericin, talcum-powder, doxycycline, silver-nitrate and control. Agents were administrated through left thoracotomy, rats sacrificed twelve-days after.

Results: Highest ratio of collagen fibers was observed in sericin group, and the intensity was higher than talcum-powder group ($p<0.05$). Compared to silver nitrate, sericin group displayed better mesothelial reaction, and multi-layer mesothelium was also better ($p<0.05$). Foreign body reaction and emphysema were less frequent in sericin group ($p<0.05$). The presence of biological tissue in parenchyma was less prominent in sericin group ($p<0.05$). Foreign body reaction on thoracic wall was less common in sericin group ($p<0.05$). Presence of biological tissue glue in thoracic wall was less prominent in sericin group ($p<0.05$).

Glomerular degeneration was lower in sericin group compared to the silver nitrate group ($p<0.05$), and tubular degeneration was less common in sericin group than talcum group ($p<0.05$). Pericarditis was less common in sericin group compared to the other groups ($p<0.05$).

Conclusion: As an intrinsic, natural glue protein, sericin protects the lung parenchyma and tissues, and its glue-like characteristics enable pleurodesis. The success of sericin in pleurodesis was demonstrated in the present study based on investigations of the pleurae. Being cost-effective and better tolerated agent associated with a low potential of side effects, sericin is more effective, less expensive and provides more lung parenchyma protection.

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¿Es el nuevo agente pleurodésico sericina más efectivo para pleurodesis que el polvo de talco, la doxiciclina y el nitrato de plata?

RESUMEN

Introducción: La utilidad de la sericina como agente pleurodésico se ha descrito previamente. El objetivo de este estudio es evaluar el éxito, efectividad, tolerabilidad y efectos secundarios de la pleurodesis con sericina.

Palabras clave:

Sericina
polvos de talco

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Abbreviations: Dox, Doxycycline; TP, talcum powder; SN, silver nitrate.

Doxiciclina
 Nitrate de plata
 Pleurodesis
 Fibrosis
 Pleura
 Parénquima
 Coste-efectivo
 Efectos secundarios

Métodos: Ratas adultas albinas Wistar de 12 semanas ($n = 60$) se dividieron en cinco grupos de tratamiento: sericina, polvos de talco, doxiciclina, nitrato de plata y control. Los agentes se administraron por toracotomía izquierda. Las ratas se sacrificaron 12 días después.

Resultados: se observó la mayor proporción de fibras de colágeno en el grupo de sericina, con intensidad superior a la del grupo de talco ($p < 0,05$). Comparado con el nitrato de plata, el grupo de sericina mostró mejor reacción mesotelial y mejor mesotelio multicapa ($p < 0,05$). La reacción a cuerpo extraño y el enfisema fueron menos frecuentes en el grupo de sericina ($p < 0,05$). Se halló menor cantidad de tejido biológico en el parénquima en el grupo de sericina ($p < 0,05$). La reacción a cuerpo extraño en la pared torácica fue menos frecuente en el grupo de sericina ($p < 0,05$). Se halló menor cantidad de adhesivo tisular de origen biológico en la pared torácica en el grupo de sericina ($p < 0,05$).

La degeneración glomerular fue menor en el grupo de sericina en comparación con el grupo de nitrato de plata ($p < 0,05$), y la degeneración tubular fue menos frecuente en el grupo de sericina que en el grupo de talco ($p < 0,05$). También la pericarditis fue menos frecuente en el grupo de sericina en comparación con los otros grupos ($p < 0,05$).

Conclusión: Como proteína adhesiva natural intrínseca, la sericina protege al parénquima pulmonar y a los tejidos, de modo que sus características adhesivas son adecuadas para la pleurodesis. En este estudio se demuestra la utilidad de la sericina en la pleurodesis gracias a investigaciones de la pleura. Siendo un agente más coste-efectivo y mejor tolerado, la sericina es más efectiva, más barata y proporciona mayor protección del parénquima pulmonar.

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Introduction

The cocoon shell of silkworms is composed of two natural proteins, called *fibroin* and *sericin*.¹ Sericin is a natural, gum-like protein that holds fibroin polymers together and is a by-product of the textile industry with a low cost-profile.¹ The removal of sericin from silk fibroin is accomplished using a specialized process called “degumming”.² Sericin has many applications, such as an anti-aging, antioxidant, and antimicrobial agent, as well as an additive to cell culture media.^{3–7} Sericin also has a healing effect on skin wounds and enhances the proliferation of fibroblasts.^{8,9}

Many substances have been used for pleurodesis; however, talcum powder (TP), doxycycline, and silver nitrate (SN) appear to be most commonly used agents. In the literature, Yazicioglu et al. have demonstrated the usefulness of sericin as a pleurodesis agent.¹⁰ However, to our knowledge, there is not any comparative study regarding sericin pleurodesis with other agents. This study aims to compare sericin pleurodesis with the other currently available agents regarding efficacy and potential side effects.

We hypothesized that sericin could be used as a pleurodesis agent if it provides to have higher effectiveness and a lower potency for side-effects compared to other agents. As the cost of production is lower with sericin, sericin pleurodesis may significantly reduce treatment and hospital costs. A profile that consists of high effectiveness, low potential for side-effects and reduce costs will ultimately increase patient comfort and satisfaction, as well as reducing healthcare spending. Our second hypothesis was that since sericin is a more cost-effective product, the use of sericin would still reduce treatment costs in if it proves to have similar pleurodesis effectiveness and side-effect profile compared to the other agents. If a similar level of effectiveness can be achieved at a lower cost, what would be the rationale for using more expensive agents? This study aims to demonstrate the effectiveness and side-effect profile of sericin in comparison to the other agents and investigate the potential areas of use for sericin as a less expensive, readily available and cost-effective product.

Materials and methods

Sericin powder was purchased from Xi'an-Lyphar-Biotech Co., Ltd., Xi'an, China; the talcum powder was purchase from NovatechSA (Steritalc®), Marseille, France, and the doxycycline was

purchased from Actavis (Tetradox® 100 mg capsule), Istanbul, Turkey. The silver nitrate was prepared from 10 g of crystal SN and to obtain 0.5% SN solution, 0.5 mg of SN was weighed and added to 100 mL of distilled water.

It was important to determine the appropriated dose of administration of agents. A study previously conducted by Yazicioglu et al. used 30 mg sericin in rats and obtained successful results.¹⁰ The administrated dose of TP in adults varied between 4 and 5 g in previous studies.¹¹ Thus, a dose of 17 mg was estimated to be appropriate for rats weighing approximately 250 ± 30 g. The administrated dose of doxycycline in adults varied between 500 and 600 mg in previous studies.¹² Hence, a dose of 2.6 mg was estimated to be appropriate for rats. Based on the previous literature, the dose to be administered for 0.5% SN solution was 30 mL for adults, while a dose of 0.45 mL/kg was administrated from 0.5% SN solution.¹³

Animals

The animal study received approval from the Ethics Committee of Kobay AS (Protocol Number-203/2016). Adult, male, 12-week-old Wistar albino rats, weighing between 220 and 290 g were used in this study ($n = 60$). The rats were randomly and equally divided into five groups: the sericin group, TP group, doxycycline group, SN group and control group. All the animals received human care in accordance with Turkish Government Animal Protection and Management Law.

Technique

The sericin powder was divided into 30-mg packs; TP was divided into 17-mg packs, doxycycline was divided into 2.6-mg packs, and added into Eppendorf-tubes. Silver nitrate, 0.45 mL/kg, was administered in liquid form. The operation and post-operative conditions of rats was performed following instructions previously described by Yazicioglu et al.¹⁰ All the rats were weighed before surgery and before sacrificed, and the respective body weights were recorded.

The rats were sacrificed at postoperative day 12; cardiac puncture was the preferred method. The left hemithorax was removed en-bloc, the lungs were fully expanded by instilling 10% formaldehyde solution, and the trachea was ligated. Subsequently, the entire

hemithorax with the heart and additionally the liver and kidney were removed and submerged in a 10% formaldehyde solution.

Pathological examination

For microscopic evaluation, representative tissue sections of the pleura, parenchyma, thoracic wall, liver, kidney, and heart were prepared. The tissue samples were processed through a graded mixture of alcohol and xylene, and embedded in paraffin blocks; 6- μ m tissue sections were obtained and stained with hematoxylin and eosin (HE) and Masson's trichrome. The pathologist (investigator F.D.) was kept blind to the study groups. Particular attention was given to the parameters of the tissues; collagen deposition was evaluated using Masson's trichrome staining (MTS).

In the pathological evaluation of the tissues a semi-quantitative method was preferred. The parameters, such as foreign body reaction, presence of biologic glue, emphysema, organizing fibrin on pleura, and potential side-effects were classified as "none" or "yes". The pathological slides were graded for inflammation/cellularity and scored using the following system developed by Hurewitz et al., and classified as "none", "mild", "moderate", and "severe".¹⁴ The degree of fibrosis was scored using the following system developed by Vargas et al., and classified as "none", "mild", "moderate", and "severe".¹⁵ The evaluation of Masson's trichrome staining was classified as "none", "sparse", and "dense". Collagen fibers were classified as "sparse" if the fibers are thin, sparse and in small number, and as "dense" if the fibers are thick and widespread.

Statistical analyses

The pathologic data were expressed using statistical analyses, which were conducted using the SPSS statistical software package (version 17.0, SPSS, Chicago, IL, United States). The one-way ANOVA test was used to analyze the potential differences between study groups. In cases where a difference was detected between the groups, Levene-test was used to check the differences between the variances and to identify which group was the source of difference. The variances were not equal when p values were <0.05 based on the Levene-tests, and the Tamhane's and Dunnett's t -test were used in such cases. Variances were considered equal when p values were <0.05 based on Levene-test, and Scheffe-test was used in such circumstances. For most of the parameters included in the statistical analyses, the Levene-test result was $p < 0.05$. Thus, the variances were found to be unequal, and the Tamhane's and Dunnett's t -test were used. Based on Scheffe, Tamhane's and Dunnett's t -tests, p values of <0.05 were considered statistically significant.¹⁶

Results

One animal in each of the doxycycline and TP groups died within the first 24 h following surgery. The remaining 58 rats completed this study and were sacrificed on the 12th day. The body weights of rats were compared between two-time points. While all rats in the sericin, doxycycline and control groups gained weight, three rats in TP group (27.3%) and four rats in SN group (33.3%) lost weight during the postoperative period. The mean weight gain among rats in control, doxycycline and sericin groups was 16.1 g (range: 7–30 g), 15.7 g (range: 5–50 g) and 33.7 g (range: 13–60 g), respectively. When the groups were compared in terms of mean weight, the procedure was found to be better tolerated by the rats in sericin group, and the highest level of weight gain was recorded in this group. The Scheffe's test indicated that the mean weight gain in the sericin group was significantly higher compared to other groups ($p < 0.05$).

The evaluation of lung parenchyma and visceral pleura

Foreign body reaction did not occur in any rat in the sericin group, however, nine (81.8%) and four (36.4%) rats showed foreign body reaction in the TP and dox groups, respectively. The statistics indicated that there were significant relations between the sericin group and TP, dox groups ($p < 0.05$). Multi-layer mesothelium was better in the sericin group compared to SN group ($p < 0.05$). Emphysema were less frequent in the sericin group compared other groups ($p < 0.05$). Biological glue was not present in any of rats in the sericin group and there was a significant relation between sericin group and other groups ($p < 0.05$). Results in this category were detailed in Table 1.

The evaluation of parietal pleura and thoracic wall

Foreign body reaction and biological glue were not present in any rat in the sericin group ($p < 0.05$). However, fibrosis in parietal pleura was more common in dox and SN groups compared to the sericin group ($p < 0.05$). Results in this category were detailed in Table 2.

The evaluation of collagen fibers of parietal and visceral pleura

Masson's trichrome staining (MTS) was used to evaluate the collagen fibers in the pleurae. In the evaluation of visceral pleura, dense collagen fibers were observed in five (45.5%), nine (75%), 10 (90.9%) and 11 rats (91.7%) in the TP, SN, dox and sericin groups, respectively. The Dunnett's t -test showed that the relationship of the sericin group with TP group was significant ($p < 0.05$). The evaluation of MTS of pleurae was described in Table 3. The microscopic examination of parenchyma, pleura, and thoracic wall were demonstrated in Figs. 1 and 2.

The evaluation of liver, kidney, heart, and pericardium

Glomerular and tubular degeneration was less common in the sericin group compared to the SN and TP groups, respectively ($p < 0.05$). Pericarditis was less common in the sericin group compared to the dox and SN groups ($p < 0.05$). However, both pyknotic nuclei and periportal inflammation were more common in the sericin group ($p < 0.05$). The evaluation of liver, kidney, heart and pericardium were described in Table 4. The microscopic examination of liver, kidney, and heart with HE staining were demonstrated in Fig. 3.

Discussion

Sericin is a macromolecular protein that contains 18 amino acids, including essential amino acids. Thus, the molecular structure of sericin contains strongly polar side groups that makes easy cross-linking and copolymerization.^{6,17} Sericin has many applications, an additive on cell culture media, it has a healing effect on skin wounds and enhances the proliferation of fibroblasts.^{3,18} Tsubouchi et al. studied the effects of sericin on cultured human skin fibroblasts, and showed sericin enhances the attachment of cultured skin fibroblasts, which are considered to play important roles in the healing process.¹⁸

If sericin increases fibroblastic activity in the skin, it may also be expected to improve it in lung parenchyma and thoracic wall. Increased fibroblastic activity in the lung and thoracic wall will manifest as fibrosis and pleural adhesion. Thus, successful pleurodesis will be achieved. It is, therefore, reasonable to use this protein with intrinsic glue characteristics to adhere to the pleural layers. Yazicioglu et al. previously performed an experimental

Table 1
The evaluation of lung parenchyma and visceral pleura.

Parameters	Classification	Control (n; %)	Sericin (n; %)	Talcum powder (n; %)	Doxycycline (n; %)	Silver nitrate (n; %)	Statistically significance (A vs. B (Test method; p value))	Explanations
Foreign body reaction	None	12; 100%	12; 100%	2; 18.2%	7; 63.6%	11; 91.7%	Sericin vs. TP (Tamhane; $p < 0.05$, $p = 0.001$), TP vs. SN (Tamhane; $p < 0.05$, $p = 0.001$), TP vs. Control (Tamhane; $p < 0.05$, $p = 0.001$) Sericin vs. TP (Dunnet's <i>t</i> -test; $p < 0.05$, $p = 0.001$), Sericin vs. Dox (Dunnet's <i>t</i> -test; $p < 0.05$, $p = 0.024$)	
	Yes	0; 0%	0; 0%	9; 81.8%	4; 36.4%	1; 8.3%		
Inflammatory reaction of lung parenchyma	None	11; 91.7%	0; 0%	2; 18.2%	0; 0%	0; 0%	Control vs. SN (Tamhane; $p < 0.05$, $p = 0.001$) Control vs. Dox (Tamhane; $p < 0.05$, $p = 0.001$) Control vs. TP (Tamhane; $p < 0.05$, $p = 0.001$) Control vs. Sericin (Tamhane; $p < 0.05$, $p = 0.001$)	The number of subjects in some groups was insufficient for statistical analysis; groups with moderate and severe inflammation were combined. Statistical analyses were performed based on the groups determined with no inflammation, mild inflammation, moderate + severe inflammation in the parenchyma.
	Mild	1; 8.3%	3; 25%	0; 0%	0; 0%	3; 25%		
	Moderate	0; 0%	3; 25%	2; 18.2%	0; 0%	2; 16.7%		
	Severe	0; 0%	6; 50%	7; 63.6%	11; 100%	7; 58.3%		
Mesothelial reaction	None	8; 66.7%	0; 0%	0; 0%	0; 0%	0; 0%	Control vs. Dox (Tamhane; $p < 0.05$, $p = 0.007$) Control vs. TP (Tamhane; $p < 0.05$, $p = 0.03$) Control vs. Sericin (Tamhane; $p < 0.05$, $p = 0.007$) Sericin vs. SN (Dunnet's <i>t</i> -test; $p < 0.05$, $p = 0.018$) Sericin vs. Control (Dunnet's <i>t</i> -test; $p < 0.05$, $p = 0.001$)	The number of subjects in some groups was insufficient for statistical analysis; groups of without mesothelial reaction and one-layer mesothelium were combined, groups of multi-layer mesothelium and mesothelial proliferation were combined.
	One-layer mesothelium	0; 0%	0; 0%	1; 9.1%	0; 0%	5; 41.7%		
	Multi-layer mesothelium	4; 33.3%	12; 100%	0; 0%	11; 100%	5; 41.7%		
	Mesothelial proliferation	0; 0%	0; 0%	10; 90.9%	0; 0%	2; 16.7%		
Emphysema	None	12; 100%	10; 83.3%	2; 18.2%	4; 36.4%	7; 58.3%	Sericin vs. TP (Tamhane; $p < 0.05$, $p = 0.008$) Sericin vs. TP (Dunnet's <i>t</i> -test; $p < 0.05$, $p = 0.001$), Sericin vs. Dox (Dunnet's <i>t</i> -test; $p < 0.05$, $p = 0.027$)	
	Yes	0; 0%	2; 16.7%	9; 81.8%	7; 63.6%	5; 41.7%		

Table 1 (Continued)

Parameters	Classification	Control (n; %)	Sericin (n; %)	Talcum powder (n; %)	Doxycycline (n; %)	Silver nitrate (n; %)	Statistically significance (A vs. B (Test method; p value))	Explanations
Presence of biologic glue	None	12; 100%	12; 100%	2; 18.2%	6; 54.5%	6; 50%	Sericin vs. TP (Tamhane; $p < 0.05$, $p = 0.001$) Sericin vs. TP (Dunnet's <i>t</i> -test; $p < 0.05$, $p = 0.001$) Sericin vs. Dox (Dunnet's <i>t</i> -test; $p < 0.05$, $p = 0.018$) Sericin vs. SN (Dunnet's <i>t</i> -test; $p < 0.05$, $p = 0.007$)	
	Yes	0; 0%	0; 0%	9; 81.8%	5; 45.6%	6; 50%		
Fibrosis of visceral pleura	None	10; 83.3%	0; 0%	2; 18.2%	0; 0%	0; 0%	Control vs. Dox (Tamhane; $p < 0.05$, $p = 0.001$) Control vs. TP (Tamhane; $p < 0.05$, $p = 0.001$) Control vs. Sericin (Tamhane; $p < 0.05$, $p = 0.001$) Control vs. SN (Tamhane, $p < 0.05$; $p = 0.001$)	The number of subjects in some groups was insufficient for statistical analysis; groups with moderate and severe fibrosis were combined. Statistical analyses were performed based on the groups determined without fibrosis, mild fibrosis, moderate + severe fibrosis in the visceral pleura.
	Mild	2; 16.7%	2; 16.7%	0; 0%	0; 0%	4; 33.3%		
	Moderate	0; 0%	1; 8.3%	1; 9.1%	1; 9.1%	5; 41.7%		
	Severe	0; 0%	9; 75%	8; 72.7%	10; 90.9%	3; 25%		
Organizing fibrin on visceral pleura	None	12; 100%	5; 41.7%	4; 36.4%	0; 0%	4; 33.3%	Control vs. Dox (Tamhane; $p < 0.05$, $p = 0.001$) Control vs. TP (Tamhane; $p < 0.05$, $p = 0.019$) Control vs. Sericin (Tamhane; $p < 0.05$, $p = 0.024$) Control vs. SN (Tamhane; $p < 0.05$, $p = 0.007$) Sericin vs. Control (Dunnet's <i>t</i> -test; $p < 0.05$, $p = 0.02$) Sericin vs. Dox (Dunnet's <i>t</i> -test; $p < 0.05$, $p = 0.047$)	
	Yes	0; 0%	7; 58.3%	7; 63.6%	11; 100%	8; 66.7%		

Abbreviations: Dox, doxycycline; TP, talcum powder; SN, silver nitrate.

Table 2
The evaluation of parietal pleura and thoracic wall.

Parameters	Classification	Control (n; %)	Sericin (n; %)	Talcum powder (n; %)	Doxycycline (n; %)	Silver nitrate (n; %)	Statistically significance (A vs. B (Test method; p value))	Explanations
Foreign body reaction	None	12; 100%	12; 100%	1; 9.1%	5; 45.6%	12; 100%	Sericin vs. TP (Tamhane; $p < 0.05$, $p = 0.001$) SN vs. TP (Tamhane; $p < 0.05$, $p = 0.001$) Control vs. TP (Tamhane; $p < 0.05$, $p = 0.001$) Sericin vs. TP (Dunnet's t -test; $p < 0.05$, $p = 0.001$) Sericin vs. Dox (Dunnet's t -test; $p < 0.05$, $p = 0.001$)	
	Yes	0; 0%	0; 0%	10; 90.9%	6; 54.5%	0; 0%		
Inflammatory reaction of thoracic wall	None	12; 100%	3; 25%	0; 0%	0; 0%	1; 8.3%	Control vs. Sericin (Tamhane; $p < 0.05$, $p = 0.004$) Control vs. TP (Tamhane; $p < 0.05$, $p = 0.001$) Control vs. Dox (Tamhane; $p < 0.05$, $p = 0.001$) Control vs. SN (Tamhane; $p < 0.05$; $p = 0.001$)	The number of subjects in some groups was insufficient for statistical analysis; groups with moderate and severe inflammation were combined. Statistical analyses were performed based on the groups determined with no inflammation, mild inflammation, moderate + severe inflammation in the thoracic wall.
	Mild	0; 0%	3; 25%	2; 18.2%	2; 18.2%	3; 25%		
	Moderate	0; 0%	3; 25%	5; 45.5%	1; 9.1%	3; 25%		
	Severe	0; 0%	3; 25%	4; 36.3%	8; 72.7%	5; 41.7%		
Mesothelial reaction	None	0; 0%	0; 0%	0; 0%	0; 0%	0; 0%	No statistically significance was calculated in the mesothelial reaction of thoracic wall.	
	One-layer mesothelium	12; 100%	9; 75%	6; 54.5%	6; 54.5%	8; 66.7%		
	Multi-layer mesothelium	0; 0%	3; 25%	5; 45.6%	5; 45.6%	4; 33.3%		
	Mesothelial proliferation	0; 0%	0; 0%	0; 0%	0; 0%	0; 0%		
Presence of biologic glue	None	12; 100%	12; 100%	0; 0%	10; 90.9%	11; 91.7%	TP vs. Sericin (Tamhane; $p < 0.05$, $p = 0.001$) TP vs. Dox (Tamhane; $p < 0.05$, $p = 0.001$) TP vs. SN (Tamhane; $p < 0.05$, $p = 0.001$) TP vs. Control (Tamhane; $p < 0.05$, $p = 0.001$)	
	Yes	0; 0%	0; 0%	11; 100%	1; 9.1%	1; 8.3%		
Fibrosis of parietal pleura	None	11; 91.7%	3; 25%	3; 27.3%	0; 0%	2; 16.6%	Control vs. Sericin (Tamhane; $p < 0.05$, $p = 0.012$) Sericin vs. Dox (Tamhane; $p < 0.05$, $p = 0.002$) Control vs. TP (Tamhane; $p < 0.05$, $p = 0.016$) Control vs. Dox (Tamhane; $p < 0.05$, $p = 0.001$) Control vs. SN (Tamhane; $p < 0.05$, $p = 0.001$) Sericin vs. Dox (Dunnet's t -test; $p < 0.05$, $p = 0.002$) Sericin vs. SN (Dunnet's t -test; $p < 0.05$, $p = 0.044$) Sericin vs. Control (Dunnet's t -test; $p < 0.05$, $p = 0.021$)	The number of subjects in some groups was insufficient for statistical analysis; groups with moderate and severe fibrosis were combined. Statistical analyses were performed based on the groups determined without fibrosis, mild fibrosis, moderate + severe fibrosis in the parietal pleura.
	Mild	1; 8.3%	7; 58.3%	1; 9.0%	0; 0%	0; 0%		
	Moderate	0; 0%	2; 16.7%	3; 27.3%	4; 36.4%	5; 41.7%		
	Severe	0; 0%	0; 0%	4; 36.3%	7; 63.6%	5; 41.7%		
Organizing fibrin on parietal pleura	None	12; 100%	9; 75%	8; 72.7%	6; 54.5%	10; 83.3%	No statistically significance was calculated in the organizing fibrin on parietal pleura.	
	Yes	0; 0%	3; 25%	3; 27.3%	5; 45.5%	2; 16.7%		

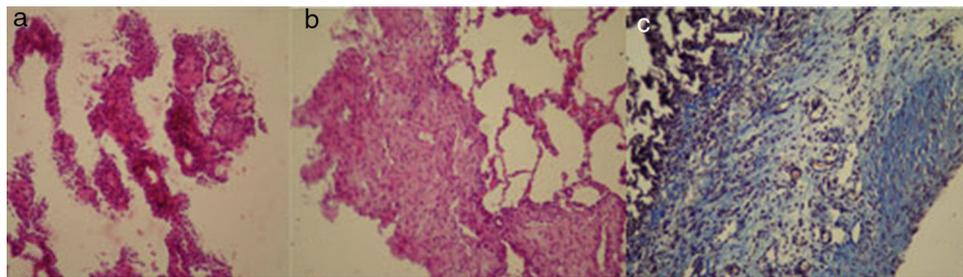
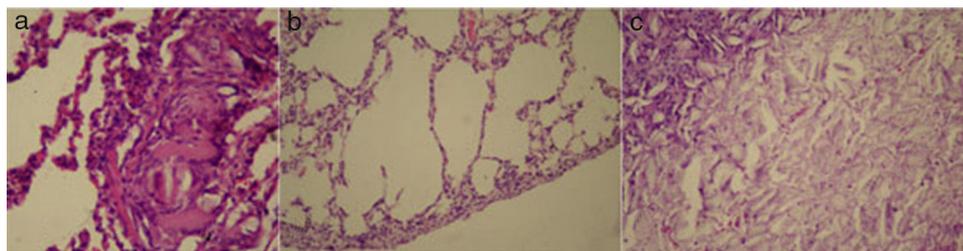
Abbreviations: Dox, doxycycline; TP, talcum powder; SN, silver nitrate.

Table 3

The evaluation of Masson's trichrome staining of parietal and visceral pleura.

Parameters	Presence of collagen fibers	Control (n; %)	Sericin (n; %)	Talcum powder (n; %)	Doxycycline (n; %)	Silver nitrate (n; %)	Statistically significance (A vs. B (Test method; p value))
Evaluation of the visceral pleura by Masson's trichrome stain	None	11; 91.7%	0; 0%	2; 18.2%	0; 0%	0; 0%	Control vs. Sericin (Tamhane; $p < 0.05$, $p = 0.001$) Control vs. TP (Tamhane; $p < 0.05$, $p = 0.004$) Control vs. Dox (Tamhane; $p < 0.05$, $p = 0.001$) Control vs. SN (Tamhane; $p < 0.05$, $p = 0.001$) Sericin vs. Control (Dunnet's <i>t</i> -test; $p < 0.05$, $p = 0.001$) Sericin vs. TP (Dunnet's <i>t</i> -test; $p < 0.05$, $p = 0.005$)
	Sparse	1; 8.3%	1; 8.3%	4; 36.3%	1; 9.1%	3; 25%	
	Dense	0; 0%	11; 91.7%	5; 45.5%	10; 90.9%	9; 75%	
Evaluation of the parietal pleura by Masson's trichrome stain	None	12; 100%	1; 8.3%	1; 9.1%	1; 9.0%	2; 16.7%	Control vs. Sericin (Tamhane; $p < 0.05$, $p = 0.001$) Control vs. TP (Tamhane; $p < 0.05$, $p = 0.001$) Control vs. Dox (Tamhane; $p < 0.05$, $p = 0.001$) Control vs. SN (Tamhane; $p < 0.05$, $p = 0.001$)
	Sparse	0; 0%	6; 50%	9; 81.8%	5; 45.5%	8; 66.6%	
	Dense	0; 0%	5; 41.7%	1; 9.1%	5; 45.5%	2; 16.7%	

Abbreviations: Dox, doxycycline; TP, talcum powder; SN, silver nitrate.

**Fig. 1.** (a) The multi-layer mesothelial reaction of visceral pleura in sericin group (HE $\times 200$); (b) fibrosis in the visceral pleura in sericin group (HE $\times 200$); (c) dense collagen fibers of visceral pleura in sericin group (Masson's trichrome $\times 200$).**Fig. 2.** (a) Foreign body reaction in the lung parenchyma in talcum powder group (HE $\times 200$); (b) emphysema of parenchyma in talcum powder group (HE $\times 200$); (c) presence of tissue glue in the lung parenchyma in talcum powder group (HE $\times 200$).

study to investigate the effectiveness of the intrapleural administration of sericin, and concluded that sericin results in an increase in fibroblastic activity and fibrosis in the visceral pleura without any significant adverse effects on lung parenchyma.¹⁰ Moreover, intrapleural sericin administration increases plasma native thiol and total thiol levels, which assist antioxidant activity and prevent free radical-induced damage.⁵

Research studies are ongoing worldwide to discover natural, inexpensive, cost-effective novel pleurodesis agents with higher effectiveness and a better safety profile.¹⁹ Each of the currently available pleurodesis agents is associated with a distinctive side effect potential. Although TP has a high level of effectiveness, it is one of the agents with the highest risk of side effects. Brant et al. performed 33 TP pleurodesis on 29 patients and reported the highest rate of complications (51.7%) after pleurodesis.²⁰

Seven patients (24.1%) suffered from major complications, such as severe hypoxemia and hypotension; two of these complicated patients died, and one death was directly attributed to adult respiratory distress syndrome (ARDS).²⁰ Rehse et al. performed 89 TP pleurodesis in 78 patients and reported 33% of respiratory complications.²¹ Beside minor complications, 11 patients (12.3%) had major complications, and one patient died following bilateral talc pleurodesis.²¹ Gonzalez et al. also reported the side effects of TP pleurodesis; the most commonly observed side effect was dyspnea ($n = 12$, 8.7%), followed by talc-related lung injury ($n = 4$, 2.9%), and respiratory deterioration ($n = 4$, 2.9%).²² In the present study, foreign body reaction (FBR) and presence of biological tissue glue (PBTG) in the lung parenchyma, and emphysema were markedly decreased in the sericin group compared to the TP group ($p < 0.05$) (Table 1). Similarly, FBR and the PBTG in the thoracic wall

Table 4
The evaluation of liver, kidney, heart and pericardium after pleurodesis.

Organ	Parameters	Classification	Control (n; %)	Sericin (n; %)	Talcum powder (n; %)	Doxycycline (n; %)	Silver nitrate (n; %)	Statistically significance (A vs. B (Test method; p value))	Explanations	
Liver	Pyknotic nuclei	None	11; 91.7%	3; 25%	6; 54.5%	0; 0%	9; 75%	SN vs. Dox (Tamhane; $p < 0.05$, $p = 0.001$) Control vs. Sericin (Tamhane; $p < 0.05$, $p = 0.004$) Control vs. Dox (Tamhane; $p < 0.05$, $p = 0.001$) Sericin vs. Control (Dunnet's <i>t</i> -test; $p < 0.05$, $p = 0.001$) Sericin vs. SN (Dunnet's <i>t</i> -test; $p < 0.05$, $p = 0.010$)	Periportal fibrosis or sinusoidal obstruction was not noted in any rat in any group. Hepatocyte necrosis was observed in one rat in the TP group (9.1%), and hepatosteatosi was noted in one rat in the dox group (9.1%). Findings of necrosis or hepatosteatosi were not detected in any other rat.	
		Yes	1; 8.3%	9; 75%	5; 45.6%	11; 100%	3; 25%			
	Periportal inflammation	None	11; 91.7%	3; 25%	4; 36.4%	11; 100%	10; 83.3%			Control vs. Sericin (Tamhane; $p < 0.05$, $p = 0.001$) Control vs. TP (Tamhane; $p < 0.05$, $p = 0.019$) Sericin vs. Dox (Tamhane; $p < 0.05$, $p = 0.001$) Dox vs. TP (Tamhane; $p < 0.05$, $p = 0.019$) Sericin vs. SN (Tamhane; $p < 0.05$, $p = 0.027$)
		Yes	1; 8.3%	9; 75%	7; 63.6%	0; 0%	2; 16.7%			
Sinusoidal dilatation	None	12; 100%	3; 25%	6; 54.5%	4; 36.4%	6; 50%	Control vs. Sericin (Tamhane; $p < 0.05$, $p = 0.004$)			
	Yes	0; 0%	9; 75%	5; 45.6%	7; 63.6%	6; 50%				
Balloon degeneration	None	12; 100%	6; 50%	3; 27.3%	1; 9.1%	9; 75%		Control vs. TP (Tamhane; $p < 0.05$, $p = 0.004$) Control vs. Dox (Tamhane; $p < 0.05$, $p = 0.001$) SN vs. Dox (Tamhane; $p < 0.05$, $p = 0.005$) Sericin vs. Control (Dunnet's <i>t</i> -test; $p < 0.05$, $p = 0.012$)		
	Yes	0; 0%	6; 50%	8; 72.7%	10; 90.9%	3; 25%				
Kidney	Glomerular degeneration	None	12; 100%	12; 100%	9; 81.8%	7; 63.6%	6; 50%		Sericin vs. SN (Dunnet's <i>t</i> -test; $p < 0.05$, $p = 0.006$)	Necrosis in the kidney tissue was not noted in any rat. Although inflammatory reaction in the perinephric tissues was noted in two rats (8.3%) in the SN group, this was not observed in any other rat.
		Yes	0; 0%	0; 0%	2; 18.2%	4; 36.4%	6; 50%			
	Tubular degeneration	None	12; 100%	12; 100%	7; 63.6%	10; 90.9%	11; 91.7%	Sericin vs. TP (Dunnet's <i>t</i> -test; $p < 0.05$, $p = 0.013$)		
Yes	0; 0%	0; 0%	4; 36.4%	1; 9.1%	1; 8.3%					
Heart	Pericarditis	None	12; 100%	11; 91.7%	9; 81.8%	0; 0%	1; 8.3%	Control vs. Dox (Tamhane; $p < 0.05$, $p = 0.001$) Control vs. SN (Tamhane; $p < 0.05$, $p = 0.001$) TP vs. SN (Tamhane; $p < 0.05$, $p = 0.001$) TP vs. Dox (Tamhane; $p < 0.05$, $p = 0.001$) Sericin vs. SN (Tamhane; $p < 0.05$, $p = 0.001$) Sericin vs. Dox (Tamhane; $p < 0.05$, $p = 0.001$)		
		Yes	0; 0%	1; 8.3%	2; 18.2%	11; 100%	11; 91.7%			

Abbreviations: Dox, doxycycline; TP, talcum powder; SN, silver nitrate.

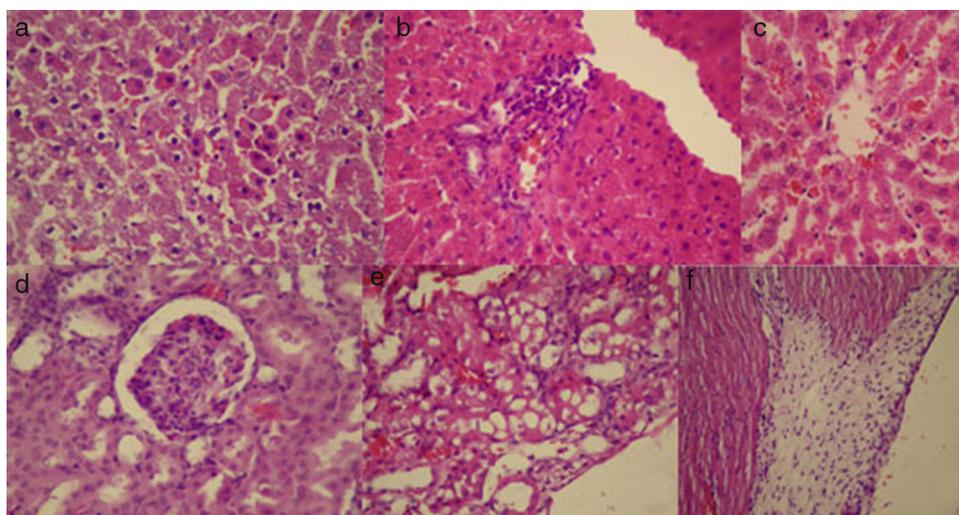


Fig. 3. (a) Pyknotic nuclei in hepatocytes in doxycycline group (HE $\times 400$); (b) periportal inflammation of liver tissue in sericin group (HE $\times 400$); (c) sinusoidal dilatation of liver tissue in sericin group (HE $\times 400$); (d) glomerular degeneration of kidney tissue in silver nitrate group (HE $\times 400$); (e) tubular degeneration of kidney tissue in talcum powder group (HE $\times 400$); (f) pericarditis in the pericardium in doxycycline group (HE $\times 400$).

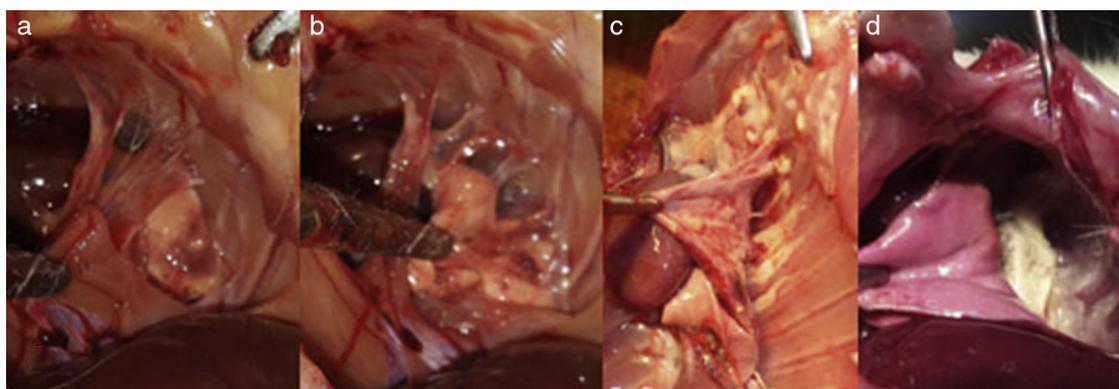


Fig. 4. (a, b) The pleural adhesions and pleurodesis success of sericin; (c, d) the accumulation of talcum powder in the thoracic wall and mediastinum after talcum pleurodesis.

were significantly lower in the sericin group compared to the TP group ($p < 0.05$) (Table 2). All these favorable outcomes achieved with sericin may be attributed to the fact that sericin is a natural protein, while TP is produced from the *hydrous magnesium silicate*. However, the histopathological or macroscopic findings of foreign body presence following TP pleurodesis have been previously reported in the literature. Ocak et al. previously reported a case of *Talcoma* which complicated differential diagnosis when observed in pleura eight years after pleurodesis.²³ In this study, the presence of foreign body was macroscopically confirmed during the sacrifice of the rats in the TP group (Fig. 4). As sericin is an intrinsic protein, it has high efficacy regarding protecting the parenchyma and other tissues. Therefore, it is consistent with the literature to expect sericin pleurodesis to result in rare side effects.^{10,24} The evaluation of the visceral and parietal pleurae by MTS showed that the collagen fibers in the sericin group were more intense compared to the TP group (Table 3). Therefore, the effectiveness of sericin in pleurodesis was higher than that of talcum powder.

Doxycycline is an antibiotic which is metabolized by the liver and excreted is from the kidneys. Previously reports indicate that doxycycline has a high level of effectiveness as a pleurodesis agent, but it has various side effects on lung tissue. Miller et al., in a study that compared doxycycline with TP, erythromycin, and diazepam pleurodesis, reported that there was a trend to higher mortality in the doxycycline-treated animals.²⁵ The doxycycline pleurodesis group had eight rabbits; three of them (38%)

died before end of the study, and the necropsies revealed severe inflammatory reactions without gross evidence of hemothorax.²⁵ In the present study, the highest rate of severe inflammation, both in lung parenchyma and the thoracic wall, was observed in the rats in the doxycycline group (Tables 1 and 2). While Miller et al. did not report any complications of hemothorax, Guo et al. presented hemothorax adverse effects after doxycycline pleurodesis in another rabbit study.²⁶ According to gross examinations of sacrificed rabbits, two (28.6%) of the seven rabbits had hemothorax.²⁶ Abouzgheib et al. reviewed the efficacy and complication rates following doxycycline pleurodesis in humans; the efficacy rates ranged from 61 to 81%.²⁷ However, chest pain complications ranged from 22 to 97% and detected fever ranged from 3 to 22% of the patients.²⁷ In another research, Mitchem et al. reported a comparative rabbit study of doxycycline and TP pleurodesis; the doxycycline group had liver tissue toxicity.²⁸ In this study, both pyknotic nuclei and balloon degeneration were most commonly noted in the doxycycline group; whereas doxycycline did not cause negative pathological changes in the kidney tissue (Table 4). When the effectiveness of sericin and doxycycline in the intrapleural administration is compared, FBR, emphysema and PBTG in the parenchyma were all markedly lower in the sericin group compared to the doxycycline group ($p < 0.05$) (Table 1). Although doxycycline is an antibiotic, it is reasonable for sericin to cause fewer side effects due to its being an intrinsic protein. On the other hand, doxycycline and

Table 5

Summary of the parameters that were significantly different in sericin pleurodesis group compared to the other pleurodesis groups. The table does not consider the comparisons between sericin and control groups.

	Advantages of sericin pleurodesis	Disadvantages of sericin pleurodesis
General evaluation	(1) Weight gain of the rats in sericin group was higher compared to the rats in all other groups ($p < 0.05$).	
Lung parenchyma and visceral pleura	(2) There was no mortality in sericin group; sericin pleurodesis was well-tolerated by the rats. (3) Serizin was found to be the agent associated with the lowest cost. Sericin was 13 times more cost-effective than talcum powder. (4) Foreign body reaction in lung parenchyma was less common in sericin group compared to the talcum powder and doxycycline groups ($p < 0.05$). (5) Sericin group had better mesothelial reaction compared to the SN group. Multi-layer mesothelium was better in sericin group compared to SN group ($p < 0.05$). (6) Emphysema was less common in sericin group compared to the talcum powder and doxycycline groups ($p < 0.05$). (7) Presence of biological tissue glue in lung parenchyma was decreased in sericin group compared to all other pleurodesis groups ($p < 0.05$).	(1) Fibrin organization in visceral pleura was better in doxycycline group compared to the sericin group ($p < 0.05$).
Parietal pleura and thoracic wall	(8) Sericin caused less foreign body reaction on the thoracic wall compared to talcum powder and doxycycline ($p < 0.05$). (9) Presence of biological tissue glue on thoracic wall was lower in sericin group compared to the talcum powder group ($p < 0.05$).	(2) Fibrosis in parietal pleura was more common in doxycycline and SN groups compared to the sericin group ($p < 0.05$).
Evaluation of the visceral and parietal pleurae by Masson's trichrome stain	(10) Evaluation of the visceral pleura by Masson's trichrome stain demonstrated that collagen fibers were denser in sericin group compared to the talcum powder group ($p < 0.05$).	(3) Pyknotic nuclei were more common in sericin group compared to SN group ($p < 0.05$).
Liver		(4) Periportal inflammation was more in sericin group compared to the SN and doxycycline groups ($p < 0.05$).
Kidney	(11) Glomerular degeneration was less common in sericin group compared to the SN group ($p < 0.05$). (12) Tubular degeneration was less common in sericin group compared to the talcum powder group ($p < 0.05$).	
Heart	(13) Pericarditis was less common in sericin group compared to the doxycycline and SN groups ($p < 0.05$).	

Abbreviation: SN, silver nitrate.

sericin pleurodesis are similar regarding the effectiveness and success. Sericin has more protective characteristics than doxycycline, and these two agents have comparable effectiveness in pleurodesis.

Silver nitrate pleurodesis is also a commonly used method, while there are previous reports on side effects. Terra et al. reported SN pleurodesis side effects; 60 patients were included, and 199 adverse effects were observed, including 23 serious adverse effects.²⁹ Hypoxia was the most frequently observed symptom; but one patient died, possibly related to pleurodesis.²⁹ The other side-effects include kidney injury, elevated serum creatinine and liver enzymes, chest pain, and embolic events.²⁹ In this study, the highest level of glomerular degeneration in the kidneys was observed in the SN group (Table 4). In another study, Vargas et al. reported the comparative results of SN versus TP pleurodesis in rabbits; hemothorax was observed in the 4.3% of SN pleurodesis group without hemothorax in the TP group.³⁰ In this study, hemothorax was not among the observed complication in either of the groups. When the effectiveness of sericin and SN pleurodesis was compared, the mesothelial reaction was found to be better in the sericin group, and multi-layer mesothelium was markedly improved in the sericin compared with the SN group ($p < 0.05$) (Table 1). Thus, sericin pleurodesis was considered as a more successful method compared with SN pleurodesis, and sericin pleurodesis accelerates fibrosis by increasing mesothelial reaction. Additionally, the PBTG in parenchyma was significantly lower in the sericin group compared to the SN group ($p < 0.05$) (Table 1).

Evaluations of the liver, kidney, heart and pericardial tissues demonstrated that sericin had a lower toxic potential. Glomerular degeneration was significantly lower in the sericin group compared

to the SN group ($p < 0.05$), and tubular degeneration was markedly lower in the sericin group compared with the TP group ($p < 0.05$) (Table 4). Pericarditis were also significantly less common in the sericin group compared to the doxycycline and SN groups ($p < 0.05$) (Table 4). Sericin pleurodesis had no side effect on the heart and kidney tissues. As a surprising finding, pyknotic nuclei formation in the sericin group was higher compared with the SN group, and periportal inflammation in the sericin group was higher compared with the doxycycline and SN groups ($p < 0.05$) (Table 4). Pyknosis is the irreversible consolidation of chromatin in the nucleus of a cell undergoing necrosis or apoptosis.³¹ The molecular basis of pyknosis is proposed to come from the internucleosomal rotational angle freedom that permits the internucleosomal sharing of basic histone tails of adjacent nucleosomes and nucleofilaments.³¹ Sericin may have dose-dependent effects on the liver. On the other hand, it is still unclear whether sericin is absorbed from the pleura and its metabolism also remains to be clarified. Although sericin was demonstrated to have several advantages over the other currently used pleurodesis agents, we believe that a large-scale project should be performed to clarify the pathological effects of sericin and doxycycline on liver tissue. Table 5 summarizes the advantages and disadvantages of sericin pleurodesis.

The cost profile of pleurodesis agents is another debatable point; inexpensive agents are needed. Sericin is an effective, natural macromolecular protein, and inexpensive when compared with other agents. Sericin offers cost-effectiveness, as it is a by-product and widely available in the textile industry. In this study, the cost of talcum powder per one rat was estimated as 0.29 dollars. On the other hand, the cost of sericin per one rat was estimated as 0.021 dollars, almost 13 times cheaper. The cost of sericin pleurodesis

was also estimated to be lower compared to the doxycycline and SN groups.

The limitation of this study was we did not compare sericin to bleomycin pleurodesis due to bleomycin being a cytotoxic agent, in contrast to TP, doxycycline, and SN, which are sclerosant agents. In the examination of inflammatory reaction in the lung parenchyma and thoracic wall, similar inflammation findings were observed in the sericin-treated group and the groups treated with other sclerosing agents. In the examination of inflammatory reactions, no statistically significant difference was found among the pleurodesis groups (Tables 1 and 2). In addition, the examination of mesothelial reactions in the visceral and parietal pleura did not show a statistically significant difference between sericin-treated group and the groups treated with other sclerosing agents. In both categories, sericin pleurodesis achieved results that were close to those with other sclerosing agents. Therefore, we believe the mechanism of sericin pleurodesis is similar to that of sclerosant agents. On the other hand, further studies are needed to elucidate the mechanism of sericin-induced pleurodesis. In addition, the long-term effects of sericin administration into the thoracic cavity are another researchable point.

The success of sericin as a pleurodesis agent was demonstrated by Masson's trichrome staining and the highest level of dense collagen fibers in pleura was noted in the sericin group. Sericin displayed a significantly higher level of protective characteristics on parenchyma and other tissues and a lower potential for inflammation. Finally, side effects concerning the kidneys and the heart were significantly lower in the sericin group. Therefore, being a better-tolerated, more cost-effective agent, associated with fewer side effects, the better protection of lung parenchyma and tissues, and more effective pleurodesis; sericin is more effective and cheaper than the other commercially available pleurodesis agents, and also protects lung parenchyma. The use of sericin as a pleurodesis agent will not only add value to the practice of thoracic surgery and pulmonology; sericin will also be beneficial to patients, surgeons, and physicians, as well as to the economy and society in general.

Conflict of interest

The authors declare no conflict of interest.

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