

# Cellulose nanofiber dispersion as a new submucosal injection material for endoscopic treatment: preliminary experimental study



## Authors

Toshiyuki Morisawa<sup>1</sup>, Akihiko Okada<sup>1</sup>, Yuuki Kataoka<sup>2</sup>, Sho Masaki<sup>3</sup>, Takayuki Hayashi<sup>4</sup>

## Institutions

- 1 Department of Gastroenterology and Hepatology, Osaka Saiseikai Nakatsu Hospital, Osaka, Japan
- 2 Hospital Care Research Unit, Hyogo Prefectural Amagasaki General Medical Center, Hyogo, Japan
- 3 Department of Gastroenterology and Hepatology, Kinki University, Osaka, Japan
- 4 Life Sciences R&D Department, R&D Headquarters, DKS Co. Ltd., Kyoto, Japan

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## Bibliography

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## Corresponding author

Toshiyuki Morisawa, MD PhD, Department of Gastroenterology and Hepatology, Osaka Saiseikai Nakatsu Hospital, 10-39, Shibata 2-chome, Kita-ku, Osaka City, Osaka, 530-0012, Japan  
 Fax: +81-6-63720339  
[mrsw-tsyk@kb3.so-net.ne.jp](mailto:mrsw-tsyk@kb3.so-net.ne.jp)

## ABSTRACT

**Background and aims** Although various solutions have been tested for submucosal injections during endoscopic treatment, the ideal solution has not been established. We investigated the suitability of a cellulose nanofiber (CNF) dispersion with high viscosity and thixotropy as a potential submucosal injection material for endoscopic treatment.

**Methods** We evaluated the catheter injectability and mucosa-elevating capacity of CNF dispersion compared with sodium hyaluronate (SH) solution, which has been reported to be a promising submucosal injection solution. The catheter injectability of CNF dispersion was examined under conditions equivalent to those used clinically in endoscopic treatment. The mucosa-elevating capacity of CNF dispersion was examined in porcine stomachs.

**Results** There was no significant difference between the catheter injectability of 0.4% CNF dispersion and 0.4% SH solutions; however, 0.4% CNF dispersion maintained significantly higher and longer elevation of the submucosal layer than 0.4% SH solution. A clear separation of the mucosal layer from the underlying muscle layer was achieved by injecting 0.4% CNF dispersion.

**Conclusion** This preliminary study suggests that CNF dispersion could be an ideal submucosal injection material for endoscopic treatment because of its unique high thixotropy index.

## Introduction

Endoscopic mucosal resection (EMR) is widely accepted as the standard treatment for early gastrointestinal cancer [1]. Despite the convenience of EMR, tumor recurrence after EMR has been a problem because of piecemeal resection of larger lesions. The technique of endoscopic submucosal dissection (ESD), which enables en bloc resection of a larger lesion without compromising curability, has rapidly become popular [2]. The creation of a submucosal cushion by fluid injection is essential for easy and safe EMR or ESD. It is accepted that the “ideal” solution for submucosal injection should provide a thick submucosal fluid cushion, remain in the submucosal space long enough to safely allow EMR or ESD, and preserve tissue speci-

mens to allow for precise pathologic staging. Generally, viscous and hypertonic solutions tend to produce adequate submucosal cushions, and a greater solution density provides a longer-lasting submucosal cushion [3]; however, highly viscous solutions are difficult to inject through a catheter. There is a demand for highly viscous submucosal injection materials retaining good injectability through a catheter. Of the various solutions proposed for submucosal injection, sodium hyaluronate (SH) has been reported to be the most effective. However, SH solution is quite expensive and research on the development of new submucosal injection materials is ongoing [3–5].

We have focused on a dispersion of cellulose nanofibers (CNF) as a potential new submucosal injection material. CNF is prepared by making extremely fine naturally derived cellulose

fibers using nanotechnology. Typical fibril widths are 5–20 nm with a wide range of lengths. CNF is a sustainable and renewable nanomaterial with many desirable features, including high strength, high modulus, low coefficient of thermal expansion, and high transparency. These basic properties of CNF make it an interesting material for many potential applications in medicine, such as a covering material for skin wounds or as an artificial vascular graft [6, 7].

We focused particularly on the TEMPO-oxidized cellulose single nanofiber (TOCN) dispersion. TEMPO (2,2,6,6-tetramethylpiperidin-1-oxyl) is a chemical compound which is used as a mediator in controlled radical polymerization, and has applications in biochemistry [8]. TOCN dispersion has a high gel-like viscosity in static conditions because of its strong thickening effect and also exhibits high thixotropy, i. e., it becomes less viscous when shaken or agitated, then returns to a gel or viscous state immediately upon cessation of movement. Thus, thixotropy is a reversible behavior [9].

We hypothesized that TOCN dispersion could act as an effective submucosal injection solution because of its high viscosity and thixotropy. The high viscosity of TOCN dispersion should provide a long-lasting thick submucosal cushion, and its high thixotropy could reduce its viscosity when it is injected through a catheter. The consequent decreased injection resistance would allow the operator to inject more easily during endoscopic treatment. In this study, we examined the catheter injectability and mucosa-elevating capacity of TOCN dispersion to evaluate the feasibility of using this material for submucosal injection.

## Material and methods

### Materials

The CNF concentrations of TOCN dispersions in this study were 0.35–0.45%. CNF dispersion takes a transparent gel-like form under static conditions and will become less viscous and flow over time when shaken or agitated. When movement ceases, it will return to a gel-like state almost instantly because of its high thixotropy. For analysis, 0.4% SH solution (Muco Up; Boston Scientific Corp., Natick, Massachusetts, United States) and physiological saline (isotonic sodium chloride solution; Otsuka Pharmaceutical Factory Corp., Tokushima, Japan) were used as the control and reference materials to examine the catheter injectability and mucosa-elevating capacity of the CNF dispersion.

### Subjective assessment of injectability of CNF dispersion into catheter

First, we analyzed the viscosity of physiological saline, 0.4% SH solution, and 0.35–0.45% CNF dispersions under static conditions. Under conditions equivalent to those clinically used in an endoscopic treatment, a 5-mL injection syringe (Terumo Corp., Tokyo, Japan) with a 25-gauge endoscopic injection needle (Top Endoscopic Injection Needle Super-Grip; Top Corp., Tokyo, Japan) was used. Ten adult men and 10 women were asked to inject each solution into a free catheter. The patients were asked to rate how difficult it was to perform catheter injection

of each solution using a numerical rating scale (NRS), with a higher score indicating more difficulty in injection. These patients were blinded as to whether they were injecting 0.35–0.45% CNF dispersions or the 0.4% SH solution.

### Objective assessment of the mucosa-elevating capacity of CNF dispersion in porcine stomach

Porcine stomachs were divided into pieces approximately 3 × 3 cm, and stretched flat on a corkboard with pins. Using a 2.5-mL syringe (Terumo Corp) with a 23-gauge needle (Terumo Corp), 2 mL of each solution was injected into the submucosa through the resected margin. Mucosal elevation was observed and recorded with a digital camera (CAMEDIA E-20; Olympus Corp., Tokyo, Japan) immediately and 5, 10, 20, 30, 60, 90, and 120 minutes after the injection. The height of mucosal elevation was measured from the recorded images, and was defined as the distance from the mucosal surface before injection to the top of the elevation. This experiment was repeated six times. We compared the mean height of mucosal elevation produced by each solution.

The stomachs injected with each solution were fixed in 10% neutral buffered formaldehyde solution and embedded in paraffin. Each paraffin block was sectioned and stained with hematoxylin and eosin for histological examination to evaluate the submucosal cushion and the effect of the injection on the tissue.

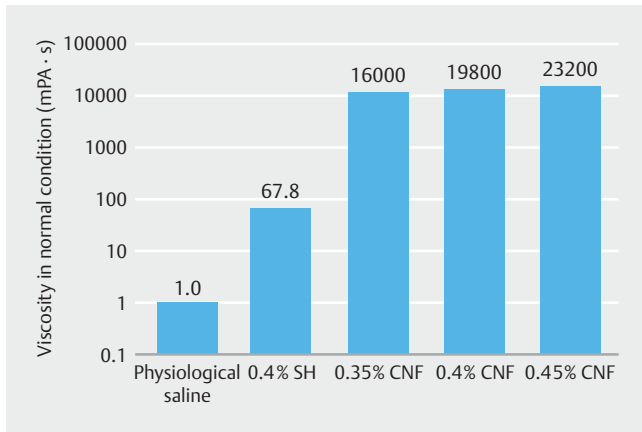
### Statistical analysis

We summarized the data using descriptive statistics. We used analysis of variance with Bonferroni's multiple comparison tests for post hoc analysis in Stata v.13.1 (Stata Corp., College Station, Texas, United States).

## Results

### Catheter injectability of CNF dispersion

► **Fig. 1** shows the static viscosity of physiological saline, 0.4% SH solution, and 0.35–0.45% CNF dispersions. The 0.35–0.45% CNF dispersions had much higher viscosity than 0.4% SH solution. The viscosity of each CNF dispersion was directly proportional to the CNF concentration. ► **Fig. 2** shows the results of the catheter injectability test. Catheter injectability revealed resistance depending on the concentration of the CNF dispersion. Compared with the 0.4% SH solution, 0.35% and 0.4% CNF dispersions showed no difficulty in catheter injectability, whereas the injectability of 0.45% CNF dispersion was clearly inferior to that of 0.4% SH solution with a statistically significant difference ( $P < 0.001$ ). The 0.4% CNF dispersion had the highest CNF concentration among the CNF dispersions that had shown no difficulty in catheter injectability compared with 0.4% SH solution.



► **Fig. 1** Viscosity of physiological saline, 0.4% sodium hyaluronate (SH), and 0.35–0.45% cellulose nanofiber (CNF) in static conditions. The viscosity of 0.4% SH solution was 67.8 mPa·s. The viscosities of 0.35%, 0.4%, and 0.45% CNF dispersions were 16 000, 19 800, and 23 200 mPa·s, respectively. The 0.4% CNF dispersion has about 300 times higher viscosity than 0.4% SH solution.

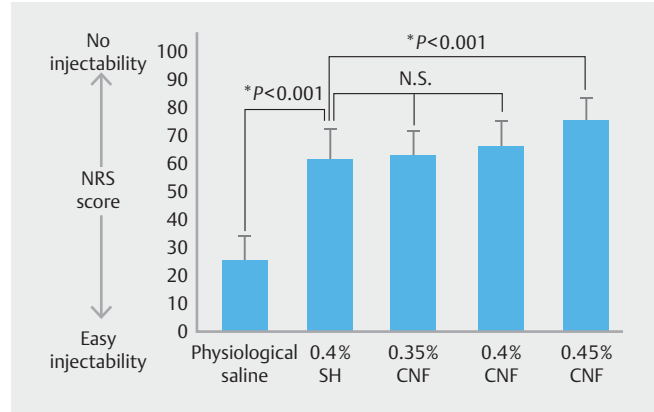
### Comparison of the ability of submucosal injection solutions to maintain mucosal elevation in porcine stomach

Based on the results of catheter injectability, the mucosa-elevating capacity in porcine stomach was examined with 0.4% CNF dispersion that had shown no difficulty in catheter injectability and had the highest viscosity. The time-dependent changes in mucosal elevation created by each solution are shown in ► **Fig. 3**. The 0.4% CNF dispersion produced a significantly higher mucosal elevation than the 0.4% SH solution at all time points after injection.

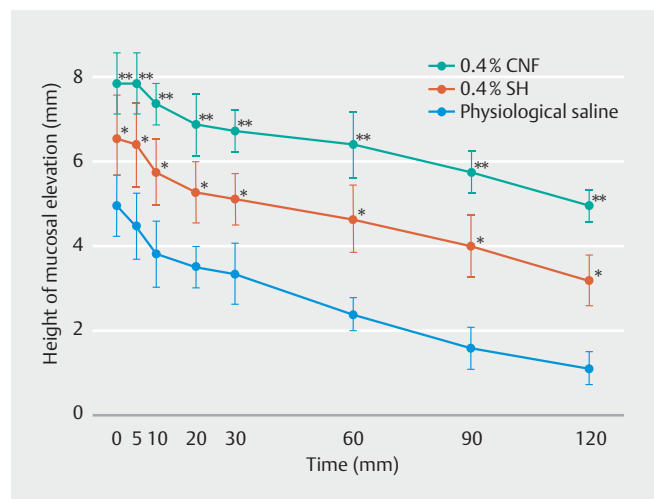
► **Fig. 4** shows typical histological images of the mucosal elevation induced by injection of 0.4% CNF dispersion. We noted accumulation of 0.4% CNF dispersion in the submucosa that separates the mucosa and the muscle. The mucosal and muscle layers were clearly separated by submucosal accumulation of 0.4% CNF dispersion, even 60 minutes after the submucosal injection. The 0.4% CNF dispersion injected into the submucosal layer caused no apparent tissue damage as seen by microscopy.

### Discussion

The results of our study demonstrated that 0.4% CNF dispersion can maintain submucosal elevation for a longer period than 0.4% SH solution. Although the viscosity of 0.4% CNF dispersion is about 300 times higher than that of 0.4% SH solution under static conditions, the catheter injectability of 0.4% CNF dispersion was equivalent to that of 0.4% SH solution. The shear stress occurring by shaking or agitating changes the viscosity of the CNF dispersion. When the operator begins to inject 0.4% CNF dispersion, it becomes less viscous and flows smoothly because of its high thixotropy index. Thus, the high thixotropy of the CNF dispersion can effectively reduce the resistance to injection and make it straightforward for the opera-

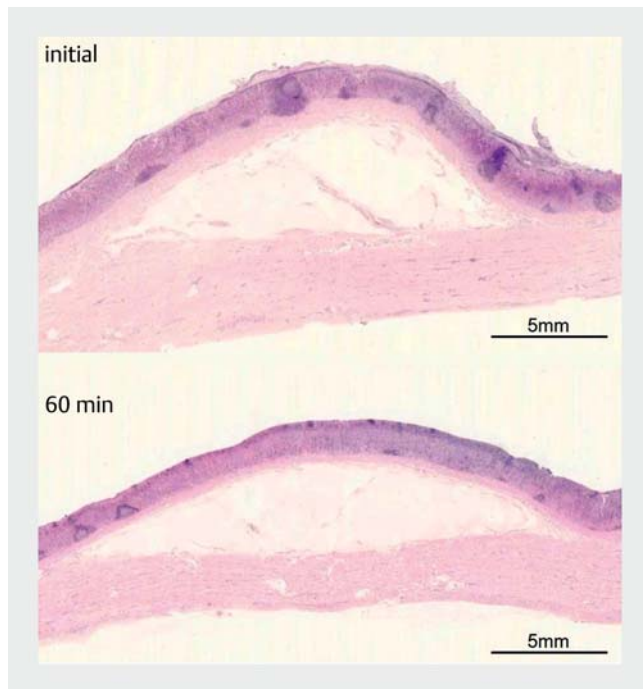


► **Fig. 2** Catheter injectability of 0.35–0.45% cellulose nanofiber (CNF) dispersion compared with 0.4% sodium hyaluronate (SH) solution. The average numerical rating scale (NRS) score for the 0.4% SH solution was 61.5 ± 11.0. The average NRS score for the 0.35% and 0.4% CNF dispersions were 62.5 ± 9.2 and 66.0 ± 9.66, respectively, and there were no statistically significant differences compared with that for the 0.4% SH solution ( $P=0.35$  and  $0.11$ , respectively). On the other hand, the average NRS score for the 0.45% CNF dispersion was significantly higher at 75.5 ± 7.97 than that for the 0.4% SH solution ( $P<0.001$ ). Furthermore, the NRS score for the 0.4% SH solution was significantly higher than that for physiological saline ( $P<0.001$ ). Individuals were asked to rate the difficulty of catheter injection of the solution using the NRS score. The results are shown as mean and standard deviation. N.S., not significant.



► **Fig. 3** Chronological changes of mucosal elevation after submucosal injection of physiological saline, 0.4% sodium hyaluronate (SH) and 0.4% cellulose nanofiber (CNF). The 0.4% CNF dispersion maintained a higher mucosal elevation compared to 0.4% SH solution. There was a statistically significant difference in the height of mucosal elevation between the 0.4% CNF dispersion and 0.4% SH solution at all points (\*\* $P<0.001$ ). 0.4% SH solution produced a higher mucosal elevation than physiological saline (\* $P<0.001$ ).

tor to inject it through a catheter. Once the injected CNF dispersion reached the submucosal layer, it returned to its original high viscosity almost instantly. This recovery of viscosity may have contributed to its ability to maintain submucosal elevation



► **Fig. 4** Histological examination of submucosal elevation immediately and 60 minutes after injection of 0.4% cellulose nanofiber (CNF) dispersion in gastric specimens (hematoxylin-eosin staining, loupe image). The mucosal layer and proper muscle layer are widely separated by the submucosal accumulation of 0.4% CNF dispersion. Sufficient accumulation of 0.4% CNF dispersion is observed in the submucosa even 60 minutes after the submucosal injection.

for a longer period compared with SH solution. The viscosity change of the CNF dispersion due to the change in shear stress was stable and consistent (► **Supplementary Fig. 1s**).

Moreover, the CNF dispersion also has a cost-benefit advantage compared with SH solution. The manufacturing cost of the CNF dispersion is much lower than that of SH solution, because cellulose is abundant in nature. The lower price of the CNF dispersion should help to reduce the cost of endoscopic treatment. These factors led us to propose that CNF dispersion could be a better submucosal injection material than 0.4% SH solution in terms of its mucosa-elevating capacity, catheter injectability, and the cost of endoscopic treatment. This is the first preliminary study to demonstrate the potential usefulness of CNF dispersion for submucosal injection.

Although the present study indicated the superior characteristics of CNF dispersion over SH solution for submucosal injection, it has some limitations. The major limitation is that the safety of the CNF dispersion in vivo has not been adequately tested. Our study was conducted in ex vivo porcine gastric mucosa. The general health, safety, and environmental aspects of CNF dispersion have recently been evaluated. The results of toxicity studies suggest that CNF dispersion is not cytotoxic and does not cause any inflammatory effects in macrophages [10].

In addition, preclinical trials of endoscopic treatment with CNF dispersion in an in vivo animal model are necessary to confirm the feasibility and safety of CNF dispersion as a new submucosal injection material. Endoscopic treatments such as EMR or ESD using TOCN dispersion as a submucosal lifting material will allow us to evaluate the formation and maintenance of mucosal lesion lifting during endoscopic treatment and the ease of mucosal resection including cutting speed, hardness, and visibility.

In conclusion, these preliminary experiments have demonstrated the superiority of CNF dispersion for injection into the submucosal layer and maintenance of mucosal thickness, illustrating the potential of the CNF dispersion as an ideal submucosal injection solution. The highly thixotropic CNF dispersion can effectively reduce the resistance to injection and maintain mucosal elevation for a long period.

## Acknowledgments

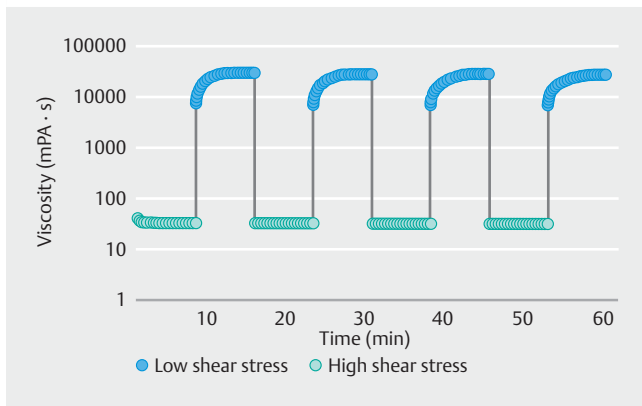
The TOCN dispersion with 0.4% CNF concentration was compounded and offered by DKS Co. Ltd., Kyoto, Japan.

## Competing interests

The authors declare that they have no conflict of interest.

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► **Supplementary Fig. 1s** The viscosity change of 0.4% CNF dispersion when shear stress is changed repeatedly. Green dots were measured viscosity values when a fixed high shear rate was applied. Blue dots were measured viscosity values when a fixed low shear rate was applied. High shear stress reduced the viscosity of CNF dispersion rapidly and the decreased viscosity value was constant. The viscosity value of the CNF dispersion decreased to about 1/300 compared to before applying shear stress because of its high thixotropy. Then, when the shear stress was decreased, the viscosity of the CNF dispersion was restored immediately and the restored viscosity value was constant and agreed with the value before applying shear stress.