Ultrasound-triggered nicotine release from nicotine-loaded cellulose hydrogel

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1. Introduction

US becomes an advanced stimulant as used in smart drug delivery systems (SDDSs) consisting of a number of state-of-the-art drug carriers [1]. The development has upgraded SDDSs as modern drug delivery systems (DDSs) due to the requirement of enhancing therapeutic efficiency by limiting drug toxicity caused by over-exposure to drugs [2,3]. Thus, the stimulation action of SDDSs is a crucial factor for designing advanced SDDSs. Therefore, researches have been expanding to identify prospective drug delivery and drug-release with some stimulants like temperature [4], light [5], magnetic field [6], pH [7], and US [8–11]. Among these stimulants, US brings advantages over the behavioral features of other DDS, since US behaves deep penetrability, inside the body manipulation ability from outside the body, non-invasiveness, and harmlessness in addition to easy and convenient use with low cost [1,10,11]. US nebulization and imaging are some key diagnostic processes due to deep penetrability to the human body [12,13]. More interestingly, US-triggered drug release and drug transportation were induced by several physical factors like oscillatory motion of surrounded fluid, acoustic streaming, cavitation, and thermal effect [1,14]. Recent researches revealed the enhancement of drug release under US irradiation as compared to conventional diffusion release and other stimulants [8–10,15,16]. On the other hand, the combination of US and biomass hydrogel matrix containing medicine behaved interestingly as an advanced DDS. For example, in the mimosa-cellulose hydrogel, the enhanced drug release was performed by US trigger due to the US promoted hydrogen bonding breakage between the medicine and the hydrogel matrix [8]. Similar US triggered behavior was observed for chitin-gallic acid medicine [9]. Also, the controlled dual drug release from the electrosprun composite matrix was achieved due to both thermal and non-thermal effects of US [15]. Therefore, US is interested in the possibility of gene delivery to cardiac, vascular, tumor, and bone tissues, transdermal release of proteins, hormones, and medical techniques like therapeutic drug systems. For particular applications, designing a
example, agave cellulose hydrogel was reported as an excellent material based hydrogels were emerging into biomedical applications. For nontoxicity in the hydrogel [20,25]. Especially, natural polysaccharide-such a matrix had extreme biocompatibility and cytocompatibility with suitable drug carrier becomes another key step to develop a SDDS [17]. Currently, the research number of drug delivery systems used for transdermal patches [18], hydrogels [16,19,20], polymer-nanoparticle composite fibers [15], multilayered capsules [21], core-shell capsules [22], and other nanocarriers [10,11,23]. In these functional materials, some of the drug carriers were used for US drug release and DDSs. Among them, hydrogel DDSs were becoming more popular as drug carriers in drug delivery and release [8,9,16,19,24]. This was due to their less toxicity by the high-water retention in the core-shell capsules [22], and other nanocarriers [10,11,23]. In these functional materials, some of the drug carriers were used for US drug release and DDSs. Among them, hydrogel DDSs were becoming more popular as drug carriers in drug delivery and release [8,9,16,19,24]. This was due to their less toxicity by the high-water retention in the polymer networking, high drug loading in the volumetric space, and the better affinity with the human body by water promoted soften role. Also, such a matrix had extreme biocompatibility and cytocompatibility with nontoxicity in the hydrogel [20,25]. Especially, natural polysaccharide-based hydrogels were emerging into biomedical applications. For example, agave cellulose hydrogel was reported as an excellent material for regenerative applications with fibroblast compatibility [25]. Also, cellulose hydrogels from purified sugarcane bagasse were successfully tested for in vivo biocompatibility using mice [26]. Nevertheless, the research numbers of such biomass hydrogels as drug carriers in US-triggered DDSs are still limited. In the present study, nicotine-loaded cellulose hydrogels are highlighted for US triggering DR system. It is known that nicotine is considered as a potential therapeutic drug for Alzheimer’s disease (AD) and Parkinson’s disease (PD) [27,28], which are known as neurodegenerative diseases. Nicotine is a natural alkaloid and is well known as an addictive drug due to smoking. Despite such a famous application, nicotine exhibits values as a medicine. Quilk et al. reviewed the protective actions of nicotine against nigrostriatal degeneration for PD [29]. The improved perception and sustained visual attention were seen for AD patients treated with nicotine [30]. Also, manganese and iron toxicities led to neurodegenerative disorders like PD, but this was completely blocked by the nicotine pretreatments [31]. However, a controlled nicotine treatment is necessary for AD and PD patients due to their extreme sensitivity to drug overdoses. Therefore, as a novel drug release system, the US-stimulated nicotine release was studied by using nicotine-entrapped hydrogel prepared with cellulose matrix. The US trigger for nicotine release is described as a prospective therapeutic application especially in neurodegenerative diseases like AD and PD. The present article includes the preparation of nicotine-loaded cellulose hydrogels and US-triggered nicotine release behavior.

### 2. Materials and methods

#### 2.1. Materials

Cotton was purchased from Kawamoto Sangyo Co., Ltd, Osaka, Japan. Lithium Chloride (LiCl), N, N-Dimethylacetamide (DMAc), Ethanol (EtOH), and Potassium Hydroxide (KOH) were purchased from Nacali Tesque, Inc. (Kyoto, Japan). Nicotine was a product of Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan). Phosphae buffered Saline was purchased from Thermo Fisher Scientific Co., Ltd. (Tokyo, Japan). Cotton was dissolved in DMAc/6 wt% LiCl solvent to obtain the required percentages of cellulose solutions. DMAc was dried in KOH for 3 days at room temperature, and LiCl was vacuum dried for 24 h at 80 °C prior to making cellulose solutions.

#### 2.2. Fabrication of nicotine-loaded cellulose hydrogels

According to our previous reports, the solvent DMAc/6 wt% LiCl was used to dissolve cotton cellulose [8,25]. By using the cellulose solution, nicotine-cellulose hydrogels were prepared according to the procedure shown in the figure (Fig. 1). First, cotton was soaked in an abundant amount of distilled water for 24 h while stirring. For 1 g of cotton, 150 ml of distilled water was used. This step promotes swelling of cellulose fibers. After 24 h, swollen fibers were separated using vacuum-induced adapter glass filtration. Then, the cotton was stirred in 150 ml ethanol for 24 h and continued the same with 150 ml DMAc. After stirring with

### Table 1

| Sample | Cellulose wt % | Nicotine wt % | Nicotine content trapped inside the washed nicotine-cellulose hydrogels (mg) | Density of Hydrogels (g/cm³) | Water content (%) | Before US exposure | After US exposure |
|--------|----------------|---------------|--------------------------------------------------------------------------|-----------------------------|------------------|-------------------|-------------------|
| N0.45  | 0.45           | 0.1           | 179 ± 15                                                                 | 1.010 ± 0.000               | 3075 ± 52        | 2924 ± 41         |
| N0.9   | 0.9            | 0.1           | 602 ± 68                                                                 | 1.016 ± 0.000               | 1971 ± 25        | 1915 ± 23         |
| N1.8   | 1.8            | 0.1           | 537 ± 68                                                                 | 1.021 ± 0.000               | 1329 ± 11        | 1290 ± 13         |
| C0.45  | 0.45           | –             | –                                                                         | 1.00 ± 0.000                | 2231 ± 70        | 3923 ± 66         |
| C0.9   | 0.9            | –             | –                                                                         | 1.013 ± 0.001               | 2005 ± 65        | 1863 ± 66         |
| C1.8   | 1.8            | –             | –                                                                         | 1.019 ± 0.001               | 1448 ± 69        | 1340 ± 79         |

**Procedure of preparing nicotine-cellulose hydrogels.**

- **Cellulose Solution (6 wt% LiCl/ DMAc)**
  - 9 g
  - Stir for 48 h at R.T.
- **Nicotine - Cellulose Solution (NCS) (6 wt% LiCl/ DMAc)**
  - Pour 10 g into 50 mm × 30 mm petri dish
  - After 24 hr, wash thoroughly to remove DMAc, LiCl and excess Nicotine
  - 24 hr phase inversion,
    - in a water vapor atmosphere
    - in a sealed container
    - at R.T.
- **Nicotine – Cellulose Hydrogel (NCH)**
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DMAC, filtered cotton was dried in a vacuum drier for 24 h to evaporate excess DMAC in cotton. Then, DMAC/6 wt% LiCl solvent was prepared by dissolving 6 g of dried LiCl in 93 ml of dried DMAC. The DMAC/6 wt% LiCl solvent and the cotton were stirred until the cotton dissolved for 1 wt% cellulose solution, at room temperature. After dissolution, the clear cellulose solution was centrifuged at 10000 rpm for 99 min to remove impurities to maintain the purity of the cellulose solution. The same procedure was applied to obtain initial cellulose solutions with 0.5 wt% and 2 wt% cellulose concentrations. Parallely, nicotine solution with 1 wt% nicotine concentration was prepared by dissolving 95% nicotine in DMAC/6 wt% LiCl solvent. After 48 h mixing of cellulose solutions with the nicotine solution in 9:1 ratio by weight, nicotine-cellulose solutions were obtained. Table 1 shows the concentration details for three samples in different cellulose concentrations of 0.45 wt%, 0.9 wt%, and 1.8 wt% nicotine-cellulose solutions. Then, the nicotine-cellulose solutions (10 g) was poured into a glass petri dish (50 mm diameter). For the gelation of the nicotine-cellulose solutions, the dish with nicotine-cellulose solution was kept in a closed container with 20 ml of distilled water. After 24 h of phase inversion under the water vapor atmosphere at room temperature, the formed cellulose hydrogel entrapped with nicotine was seen in the dish. The hydrogels having 3.4 ± 0.3 mm thickness and 29 ± 1.5 mm diameter nicotine-cellulose hydrogels were obtained for the 0.45 wt%, 0.9 wt%, and 1.8 wt% cellulose solutions with nicotine. To remove DMAC, LiCl, and unbound nicotine, the hydrogels were washed abundantly by 20 ml × 30 times of distilled water. Table 1 lists the nicotine-cellulose hydrogels samples. There were six samples in the presence and absence of nicotine having the sample name of NC0.45, NC0.9, and NC1.8 for nicotine-loaded cellulose hydrogels and C0.45, C0.9, and C1.8 in the absence of nicotine, with different cellulose concentration where 0.45 wt%, 0.9 wt%, and 1.8 wt% cellulose, respectively. Here, the nicotine concentration was fixed at 0.1 wt% in the former sample groups. Fig. 2 contains the pictures of the NC0.9 in comparison with only cellulose hydrogel of C0.9. It was appeared that the nicotine-cellulose hydrogel showed yellowish color compared to cellulose hydrogel (C0.9).

2.3. Characterization of nicotine-cellulose hydrogels

Water contents of the prepared hydrogels were calculated using the equation \( \frac{W_w - W_d}{W_i} \times 100\% \) where \( W_w \) and \( W_d \) are the weight of the hydrogel in the wet and dry state, respectively. Before the value of \( W_w \) was measured, hydrogel surface was wiped softly. Hydrogels were dried in a vacuum drier at 80 °C for 24 h to obtain the dry weight of hydrogels. Three samples from each sample category were used to ensure its repeatability [8]. Density of the nicotine-cellulose hydrogels and cellulose hydrogels were measured using the multifunctional balance (GX-200, A&D Company Limited, Japan). The densities of hydrogels were measured at 25 °C. This measurement was independently triplicated per each sample to ensure the consistency of results. To observe the cross-sections of the nicotine-cellulose hydrogels, SEM images were measured using TM3030Plus tabletop microscope (HITACHI High-Tech, Japan) for the freeze-dried hydrogels with gold sputtering on the cross-sections.

Nicotine amounts entrapped in NC0.45, NC0.9, and NC1.8 hydrogels were measured according to a reported method with few modifications [4]. Nicotine-cellulose hydrogel was cut into small pieces and stirred with 20 ml × 3 times of distilled water for 1 h, 1 h, and 24 h consecutive stirring steps allowing trapped nicotine to release to the water medium, at 40 °C. At each stirring, water was filtered and collected together. Here, the amount of trapped nicotine in the hydrogel is the nicotine amount diffused into the total 60 ml of water. The experiment was continued independently for three samples to confirm the reproducibility.

2.4. Experimental setup for US-triggered nicotine release from nicotine-cellulose hydrogels and the analysis of the hydrogel matrix after US exposure

The prepared hydrogels were exposed to US using the schematic experimental setup shown in Fig. 3. The nicotine-cellulose hydrogel was inserted into a bottle filled with 60 ml of distilled water, and then the bottle was immersed in the water-fed sonoreactor (HSR-305R, Honda Electronics Co., Ltd., Japan), 13 cm × 12.5 cm × 8 cm in size. The distance from the surface of the US transducer to the middle of the bottle was fixed at 6.5 cm, and the bottom of the bottle was adjusted in 2.5 cm from the bottom of the sonoreactor throughout the experiments. In the bottle, the nicotine-cellulose hydrogel was placed horizontally, as illustrated. The bath temperature was controlled at 25 °C using heating circular device (Lauda E100, Hansen & Co., Ltd., Japan) during the sonoreactor was running. The required US frequency and output powers were controlled with a Wave factory (15 MHz WF1943B multifunction synthesizer, NF, Japan). The generated signal amplified using high-speed bipolar amplifier (DC-1 MHz/10GVA HAS 4032, NF, Japan) was finally sent via a wave homogenizer (Honda Electronics, Japan) before transducing. In the US-triggered experiments, repeatability was confirmed by triplicating the experiment in each US condition for the respective nicotine-cellulose hydrogel.
To measure the nicotine concentration in the water filled bottle in the sonoreactor, the absorption peak of the nicotine in the 60 ml of water containing the nicotine-cellulose hydrogel was determined at 260 nm wavelength by using UV/VIS/NIR spectrophotometer (Jasco V-570, UV/VIS/NIR spectrophotometer, Jasco Corporation, Japan). This absorption peak having the characteristic band for nicotine was due to the $\pi \rightarrow \pi^*$ electron transition in pyridine ring present in nicotine [32].

Nicotine release behavior from NC0.9 hydrogel was further studied under physiological environment with phosphate buffered saline (PBS) solution at 37 °C. The same experimental system explain above was used with 60 ml PBS solution at 37 °C. Here, the absorption peak of nicotine UV–Vis spectra obtained in PBS was shifted to 258 nm wavelength.

The US effect on viscoelastic behavior on the nicotine-cellulose hydrogels was analyzed using rheometer (Physica MCR 301, Anton Paar, Austria). The measurements were taken from 0.01%–10% strain range at 1 Hz frequency at 25 °C for the storage modulus ($G'$) and the loss modulus ($G''$). Further, the values of $\tan \delta = \frac{G''}{G'}$ were recorded at each strain rate.

FT-IR analysis was carried out using the JASCO FT/IR-4100 instrument (JASCO Corporation, Japan) for the nicotine-cellulose hydrogel films before and after the US exposure. The sample films of the NC0.9 nicotine-cellulose hydrogel was used in the measurement after vacuum dried for 24 h at room temperature. The entrapment of nicotine inside the dried nicotine-cellulose hydrogel film was confirmed with the absorption peak that appeared at 260 nm in the UV–Vis spectra for the dried nicotine-cellulose hydrogel film. Then, the dried film was introduced with 2 $\mu$l distilled water drop to swell the film. The swollen film was sandwiched in between two CaF$_2$ plates (30 mm Ø × 2 mm) and sealed the opening at the circumference with a sealing tape (0.1 mm × 13 mm, SAN-EI, Japan). FT-IR spectra were obtained for the wavelength range of 4000 cm$^{-1}$–1000 cm$^{-1}$ and were smoothed using the Savitzky-Golay filter in the Spectra Manager 2.0 software of the FT-IR machine and baseline corrected using Origin 2018. A set of FT-IR spectra for the NC0.9 nicotine-cellulose hydrogel films with five different thicknesses were recorded without exposing to US. These spectra were used to obtain generalized two-dimensional correlation spectra (2D-CoS) of the OH-stretching region at 3000 cm$^{-1}$–3700 cm$^{-1}$, using 2Dshige version 1.3 software (2Dshige (c) Shigeaki Morita, Kwansei-Gakuin University, Japan, 2004–2005). Those synchronous and asynchronous 2D maps were drawn using Origin 2018, and negative correlation intensities were shaded, while positive correlation intensities were remained unshaded.

In order to identify the effect of US on the FT-IR spectra of the hydrogel film, those analyses were compared before and after the US exposure at 43 kHz/40 W for 60 min. FT-IR measurements were taken at the same point of the sample. With the correlation peaks identified from the 2D maps, the OH-stretching region was deconvoluted into Gaussian peaks using Fit peaks (Pro) in the Origin 2018 software. The correctnesses of fitted curves were ensured with the $R^2 > 0.9$.

3. Results and discussion

3.1. US-triggered nicotine release from nicotine-cellulose hydrogels

In order to investigate the US-triggered nicotine release behavior of the nicotine-cellulose hydrogels, the fabricated hydrogel was exposed to US using the experimental setup, as shown in Fig. 3. This disk shaped nicotine-cellulose hydrogel can be suggested to use external to body to administrate drug via the skin. However, further development and studies are required until the final application. In the current work, possibility to use nicotine-loaded cellulose hydrogel under US-triggered drug release and the preliminary analysis is discussed extensively. The nicotine concentration in the distilled water was measured at each US exposure time by UV–Vis absorption. The figures, Fig. 4 (a), (b), and (c) are described for the time change in nicotine release behavior of NC0.45, NC0.9, and NC1.8, respectively. Here, at different US output powers of 5 W, 10 W, 20 W, 30 W, and 40 W at 43 kHz US frequency, the US-triggered behavior was compared with the release at no US conditions in distilled water environment.

Fig. 4. Nicotine release behavior from nicotine-cellulose hydrogels prepared with different cellulose concentrations (a) 0.45 wt% (b) 0.9 wt% and (c) 1.8 wt%. US output powers ranged as 5 W, 10 W, 20 W, 30 W and 40 W at 43 kHz US frequency, at 25 °C, for 60 min. The US-triggered behavior was compared with the release at no US conditions in distilled water environment.
nicotine release in each releasing system without US. The open symbols in their plots were relevant to the release without US. For the NC0.45 (Fig. 4(a)), it was seen that the nicotine release behavior was almost the same at each US power and, poor release enhancement was seen when compared in the absence of US. Here, the release amount of nicotine became about 2 µg/ml at 60 min exposure in each of the cases for NC0.45. In contrast, the NC0.9 and NC1.8 were significant in the enhancement of US-triggered nicotine release, as given in Fig. 4(b) and (c). Here, it is important to note that the initial nicotine contents inside the respective nicotine-cellulose hydrogels were affected for the released nicotine amount during US exposure. As seen in Table 1, the amounts of nicotine entrapped in the nicotine-cellulose hydrogels were 179 µg, 602 µg, and 537 µg for NC0.45, NC0.9, and NC1.8, respectively. These differences in the entrapped nicotine amounts in the formed hydrogels with different cellulose concentrations were due to the cellulose density and matrix structure. Here, the cellulose amounts in the hydrogel films were changed when the hydrogels were formed from 0.45 wt%, 0.9 wt%, and 1.8 wt% of cellulose in the solution. As a result, the formed hydrogel films possessed different water contents, as given in Table 1. In the lower cellulose-loaded hydrogel of NC0.45, higher water was contained, while higher cellulose-loaded hydrogel (NC1.8) showed lower water content. This meant that the hydrogel film formed from lower cellulose solutions contained loose hydrogel structure having lower cellulose density. However, higher cellulose concentrations led to tight cellulose hydrogels in the structure, showing the degree of the entanglements of the cellulose chains formed the tight hydrogel matrix. In the SEM, the cross-section images of NC0.45, NC0.9, and NC1.8 in Fig. 6 had evidence of the different cellulose matrix density and structure in each hydrogel. Here, the loose hydrogel matrix was seen for NC0.45 (Fig. 6(a)), while the matrix density was increased for NC1.8 as (Fig. 6(g)). For the NC0.9 hydrogel in Fig. 6(d), the structure showed high porosity with intermediate matrix density. Therefore, the NC0.9 and NC1.8 hydrogels were capable of more space for nicotine loading in the cellulose network in the hydrogel due to the higher cellulose density and the porosity.

Moreover, the NC0.9 showed distinguishable deviations in the amounts of nicotine released over the range of US powers used from 5 W to 40 W, compared to the NC0.45 and NC1.8 under the same conditions. More interestingly, among the three nicotine-cellulose hydrogels, the highest nicotine release of 5 µg/ml at 60 min exposure was observed for NC0.9, at 40 W. Even though the NC1.8 contained considerably high amounts of nicotine inside, the responses to different US powers were not noticeable in nicotine release as shown in Fig. 4(c). This might be because of the tightest hydrogel matrix of NC1.8, as shown in Fig. 6(g). Out of the total nicotine content, the percentage amounts of the released nicotine at 60 min were measured for NC0.45 as 60%, 70%, 74% and 80% at 0 W, 5 W, 20 W and 40 W. Similar measurement was done for NC0.9 as 19%, 32%, 34% and 50% and for NC1.8 as 17%, 35%, 36% and 41%. When the US power was changed from 5 W to 40 W, the releasing percentage was increased. But this was comparatively narrow for the NC0.45 in 70%–80% and NC1.8 in 35%–41%. In fact, the US triggering effect on the nicotine releasing depended upon the hydrogel matrix. In figures, Fig. 6(b), (c), and (h), the SEM images were obtained after US irradiation, showing that the shrunk structure of the matrix was caused by the US irradiation. According to figures, Fig. 6(b) and (d), the NC0.45 was easily shrunk by the US irradiation because, in the SEM view, the loose matrix could be easily affected under the external US powers. Thus, the entrapped nicotine was easily released out even under small US powers like 5 W and 10 W. However, in the case of NC1.8, the hydrogel matrix was hardened more after US exposure, as given in Fig. 6(h), and a tight and hard wall of the cellulose matrix was observed at X2500 magnification. It was seen that the NC1.8 had the densest matrix in the samples, and thus the stimulation effects of US on the NC1.8 matrix limited the change in the cellulose wall in the matrix even at higher US powers like 40 W. Nevertheless, for the NC0.9, the triggering effect of US on the nicotine release was well-noticeable as seen in Fig. 4(c). This was because the matrix was responded in order with the changing US powers from 5 W to 40 W. Here, after the US irradiation, the porous structure of the NC0.9 was disappeared and became lamellar-structured walls as observed at higher magnification of X2500 in Fig. 6(f). This was signs for the loaded nicotine in the matrix could be released in a controlled manner accordingly at different US powers due to this lamellar structure. This is because such a layered structure of the matrix could easily respond to the US than the one relative to the hard thick wall like the NC1.8.

In Fig. 5, results of nicotine release from NC0.9 hydrogel in physiological environment of PBS was compared with the release in distilled water at 37 °C. According to the results, the release of nicotine in PBS Fig. 5(b) is lower compared in distilled water Fig. 5(a). The reason

![Fig. 5. Nicotine release behavior from NC0.9 hydrogel in (a) distilled water and (b) PBS environment at 37 °C. US at 43 kHz frequency and 5 W and 40 W output power for 60 min irradiation.](image-url)
could be the lower diffusion of nicotine in salt medium [33]. However, US was triggered the nicotine release both in distilled water and PBS while lower basal release of nicotine was shown in PBS at 37 °C than in distilled water. This ment that the US-triggered nicotine release is possible in the physiological environment as well. However, in both mediums, at 5 W and 40 W differences in nicotine release was not noticeable. Here, the reason could be the loosen cellulose hydrogel matrix at 37 °C. Due to the high temperature, the hydrogen bonds have broken thus the matrix was loosen. Further, the nicotine-cellulose interactions were also broken and thus nicotine was release easily despite the US power.

3.2. Effect of US on the nicotine-cellulose matrix

The properties of the nicotine-cellulose hydrogel matrix are crucial to consider the differences in US-triggered nicotine release for each nicotine-cellulose hydrogel. The figures, Fig. 7 (a), (c), and (e) exhibit storage modulus (G’) and loss modulus (G’’) of NC0.45, NC0.9, and NC1.8, respectively, before and after the US irradiation. According to the results, it was clearly visible that the G’ value at 0.01% strain rate
Fig. 7. (a), (c), (e) Amplitude sweep measurement of nicotine-cellulose hydrogels and (b), (d), (f) tan δ, with and without US irradiation. (a), (b) NC0.45, (c), (d) NC0.9 and (e), (f) NC1.8. US conditions were 40 W/43 kHz and irradiation time was 60 min, at 25 °C. The measurements were taken within 0.01%–10% strain rate, at 1 Hz, at 25 °C. tan δ = G''/G'
was increased when the cellulose loading was increased from 0.45 wt% to 1.8 wt%. The reason was the loose cellulose network at lower cellulose loading, like 0.45 wt%, forming a soft hydrogel. The tight hydrogel network of the hydrogels in the 1.8 wt% cellulose makes the hydrogel stiffer (Fig. 6 (g)). However, the US exposure at 40 W output power for 60 min was caused to reduce the G' of the nicotine-cellulose hydrogels at 0.01% strain. The G' values were decreased for NC0.45 from $5.9 \times 10^5$ Pa to $4.3 \times 10^4$ Pa, while for the NC0.9 from $1.8 \times 10^5$ Pa to $1.3 \times 10^5$ Pa, and for NC1.8 from $3.2 \times 10^5$ Pa to $2.4 \times 10^5$ Pa. This reduction of the G' values in their hydrogels meant that the US was transmitted through the nicotine-cellulose hydrogel matrix, and thus some internal hydrogen bonds between cellulose segments were broken upon US exposure. Considering the stimulated-nicotine release behavior and the reduction of water contents during the US triggering (Table 1), the breakage of cellulose-nicotine and cellulose-water hydrogen bonds in the matrix might be possible by the US exposure. Furthermore, the % strain rate at $\tan \delta = G''/G' = 1$, was changed to increase from 2.5% to 5.8% in the NC0.45 and in NC0.9 from 1.9% to 2.4%, which meaning that the matrix elasticity was increased in the US exposure. Therefore, this suggested the formation of inter and intramolecular hydrogen bonds in the cellulose network. However, the formation of cellulose hydrogen bonds reduced the elasticity for NC1.8 due to the dense structure. Thus, significant US effects on the viscoelastic properties were seen in the hydrogels. Elaborately, the effect of different US powers on the G' and G'' values of NC0.9 hydrogel were plotted in Fig. 8 (a). For the NC0.9 hydrogel, the G' values at the 0.01% strain rate at 0 W, 5 W, 20 W, and 40 W were $1.8 \times 10^5$ Pa, $1.5 \times 10^5$ Pa, $1.4 \times 10^5$ Pa, and $1.3 \times 10^5$ Pa, respectively. As per the results, the G' values were trending downward when the US output power was increasing. A similar tendency was observed for NC0.45 as $5.9 \times 10^4$ Pa, $5.3 \times 10^4$ Pa, $5.2 \times 10^4$ Pa, and $4.3 \times 10^4$ Pa, and for NC1.8 as $3.2 \times 10^5$ Pa, $3.1 \times 10^5$ Pa, $2.7 \times 10^5$ Pa, and $2.4 \times 10^5$ Pa, as summarized in Fig. 8 (b). This meant that when the US power was increasing, the release of nicotine and water from the cellulose matrix was augmented proportionately by the increased energy transfer to the hydrogel during the transmittance of US through it. Also, this stimulation function of US to the cellulose matrix was occurred irrespective of the differences in the cellulose concentrations in the three hydrogel systems.
However, comparing the viscoelastic properties of NC0.9 hydrogels at 25 °C and 37 °C, the $G'$ at 0.01% strain was $1.8 \times 10^5$ Pa and $6.1 \times 10^4$ Pa, respectively, without exposing to US (Fig. 9). Which means, at 37 °C the NC0.9 matrix has softened due to the high temperature. At 37 °C, the matrix strength was further decreased by the US exposure at 37 °C. The results suggest the matrix softening at high temperature and this behavior further confirms the enhanced drug release behavior at 37 °C as discussed in Fig. 5.

In order to investigate the hydrogen bonds in the nicotine-cellulose hydrogel films, FTIR spectra for the NC0.9 were measured in the presence and the absence of US (Fig. 10 (a)). Their deconvolution analysis is shown in Fig. 11. The baseline correction for the two spectra in Fig. 10 (a) was performed using base points at the same (x, y) coordinates. In the comparison, it was clearly shown an intensity reduction in the OH stretching region at 3000 cm$^{-1}$–3700 cm$^{-1}$ in Fig. 10 (a) in the absence and presence of US. Considering the OH stretching region, the intensity reduction is possible due to both water and nicotine release out from the cellulose matrix. Prior to deconvolute the OH stretching region, the possible correlations hidden in the OH stretching region were identified using 2D-CoS obtained from 2DShige software as described [34,35]. Synchronous and asynchronous 2D-CoS are shown in Fig. 10 (b) and (c), respectively. In the Synchronous map, one strong auto-peak at (3407, 3407) and two cross-peaks at (3610, 3407) and (3610, 3407) have appeared. These three peaks were positive. Further, from the asynchronous map, three positive cross-peaks at (3582, 3433), (3248, 3433), and (3100, 3433), and three negative peaks at (3433, 3582), (3433, 3248) and (3433, 3100) were recorded. Therefore, the OH stretching region of nicotine-cellulose hydrogel within 3000 cm$^{-1}$–3700 cm$^{-1}$ was identified as a combination of six peaks appeared at 3610, 3585, 3433, 3407, 3248, and 3100 cm$^{-1}$ for the positions of hydrogen bonds.

Deconvolution of the OH region of FT-IR spectra of nicotine-cellulose hydrogels was performed using Origin 2018 software according to the recent works [8,9,36]. Depending on the peaks obtained from the 2D-CoS, the OH stretching band was deconvoluted into six Gaussian peaks, and comparison was made before and after US irradiation, as
shown in Fig. 11 (a) and (b), respectively. Here, the peak 1, 2, and 4 at 3610 cm\(^{-1}\), 3585 cm\(^{-1}\), and 3407 cm\(^{-1}\) are assigned to free water in the matrix [37], free OH in the cellulose molecules [38,39], and OH stretching of the intermolecular hydrogen bonding of hydroxyl groups of cellulose [37,39,40], respectively. Peak 5 at 3248 cm\(^{-1}\) was assigned to cellulose-water as the cellulose –OH strongly bonded to water appears around 3200 cm\(^{-1}\) [40]. The peak 3 at 3433 cm\(^{-1}\) was assigned to cellulose-nicotine as this bond is less strong compared to cellulose-water because the –HO–H\(_2\)O is stronger than –HO–N in nicotine. Thus, the cellulose-nicotine peak should be at a higher wavenumber than the cellulose-water peak. According to Bailey et al., at lower water concentrations, the interaction of hydrogen bond between nitrogen atom in pyridine moieties and water is higher [41]. Therefore, the nicotine-water peak was assigned as peak 6 at 3100 cm\(^{-1}\) in the shorter wavenumber end. Furthermore, the deconvolution of the FT-IR band at 1500 cm\(^{-1}\)–1750 cm\(^{-1}\) was performed, and two hidden peaks at 1680 cm\(^{-1}\) (peak a) and 1645 cm\(^{-1}\) (peak b) were identified (Fig. 11 (c) and (d)). Here, peak a was assigned to C–N stretching of the pyridine ring [42] while peak b was assigned to –OH bending of bound water [43].

In the US effect on the OH stretching region, the FTIR intensities of the peaks 3 and 5 of OH region were greatly reduced after exposure to US for 60 min at 40 W/43 kHz US conditions. This meant that the US promoted breaking hydrogen bonds between cellulose-nicotine and cellulose-water, resulting in releasing nicotine and water. Water removal from the matrix was further proved by the intensity reduction of the peak b (Fig. 11 (c) and (d)). Further, the intensity of peak 4 in Fig. 11 (a) and (b) was increased after US irradiation, meaning the formation of intermolecular hydrogen bonds in the cellulose matrix. Collectively, the results concluded that the nicotine release behavior was stimulated by breaking hydrogen bonds of nicotine-cellulose under US irradiation.

4. Conclusion

In the present study, US-stimulated nicotine release behavior from nicotine-loaded cellulose hydrogel was studied. In NC0.9, the
entrapment of the highest amounts of nicotine in the cellulose hydrogel due to the high porous structure and the laminar-structured wall reformation acted on the efficient nicotine releasing under US trig. The combined results of 2D-CoS and deconvolution of the reformation acted on the efficient nicotine releasing under US trig.

The work reported in this paper. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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