Chemical imaging analysis of active pharmaceutical ingredient in dissolving microneedle arrays by Raman spectroscopy

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Abstract
The purpose of this study was to develop a quality evaluation method for dissolving microneedle arrays (DMNAs) and determine the spatial distribution pattern of drugs in DMNAs. Raman spectroscopy mapping was used to visualize the drug distribution in DMNAs and drug-loaded polymer films as a model. Powder X-ray diffraction (PXRD) and high-pressure liquid chromatography were also performed to characterize DMNAs. Drug-loaded polymer films and DMNAs were prepared by drying the aqueous solutions spread on the plates or casting. PXRD analysis suggested the crystallization of diclofenac sodium (DCF) in several forms depending on its amount in the sodium hyaluronate (HA)–based films. The Raman spectra of HA and DCF showed characteristic and non-overlapping peaks at 1376 and 1579 cm−1 Raman shifts, respectively. The intensity of the characteristic peak of DCF in the DCF-loaded films increased linearly with the increasing drug content in the range of 4.8 to 16.7% (DCF, w/w). Raman imaging analysis revealed a homogenous dispersion of small DCF crystals in these films. Raman imaging indicates the distribution of DCF on the surface of the DMNA needle. This work highlights the benefit of using Raman spectroscopy mapping to reveal the spatial distribution of drugs in DMNAs.

Keywords Dissolving microneedles · Raman spectroscopy · Drug distribution · Drug delivery system · Quality evaluation method

Introduction
The transdermal drug delivery system (TDDS) is an attractive alternative to conventional drug delivery methods of oral administration or injection. TDDS offers a variety of advantages, including feasible controlled delivery or sustained release of drugs, avoiding first-pass hepatic metabolism, and a patient-friendly approach [1]. However, molecular transdermal transport is limited by the skin barrier function, which forms epidermal tight junctions. Microneedle arrays (MNAs) [2] are an effective approach to deliver chemical therapeutics, biologicals, and vaccine antigens through the skin, overcoming these limitations. MNAs are minimally invasive drug delivery systems that painlessly pierce the stratum corneum (SC) to enhance molecular transdermal transport. MNA is composed of multiple micron-scale needles less than 1 mm in length, which is long enough to physically penetrate the SC, but short and narrow enough to avoid stimulating pain receptors. MNAs are categorized into four main types as follows: (1) solid MNAs [3] for skin pretreatment to enhance permeability, (2) hollow MNAs [4] for drug solution injection, (3) coated MNAs [5] with drug coating that dissolves in the skin, (4) dissolving MNAs [6] (DMNA) that encapsulate the drug and dissolve it in the skin. Each type of MNA has specific advantages and disadvantages. The first three types are mainly fabricated with non-biodegradable materials, such as silicon or metal, owing to their stiffness and molding properties [7]. The preparation of DMNAs by using certain dissolving biodegradable materials, such as hyaluronic acid [6], chondroitin sulfate [8], and carboxymethylcellulose [9], as base polymers are considered promising methods over other types of MNAs, owing to the lower risk of broken needle tip being left in the skin [10], lower production costs, better stability of loaded active pharmaceutical ingredients (APIs) [11, 12], one-step application, and ability to control the drug release profile [13].

For DMNAs, it is important to determine the spatial distribution of drugs on individual needles [14, 15]. While most
DMNAs are composed of a needle and a baseplate, only the drug encapsulated at a certain position in the needles would be efficiently delivered via dissolution after application. Methods to prepare DMNAs with appropriate drug distribution and their access have been receiving increasing attention [16]. The preparation methods include the application of certain micro-molding technologies. For example, the fabrication of needles using polymer-drug blends, followed by the addition of a baseplate by casting a drug-free formulation decreases API waste [17, 18]. These preparation methods, however, have some risks as the clinically available dose is unclear due to a possible shift of APIs into the baseplate via diffusion [19]. Despite the importance of accurate drug positioning in DMNAs, appropriate methods to detect the spatial distribution of the drug are not yet available. Preparation of model DMNAs by using a color tracer (rhodamine B) suggested a large effect of the polymer concentration in the casting solution on the distribution of the tracer [20]. High-pressure liquid chromatography (HPLC) of the drug using the collected needle parts provided only rough information on the content. Therefore, an appropriate method to analyze drug distribution on the needle is warranted.

Raman spectroscopy, which provides the molecular fingerprint by measuring the vibrational or rotational energies of chemical bonds, is a potent analytical technology for chemical identification and physical characterization of materials. It has been used as a rapid non-destructive method to analyze various pharmaceutical formulations and as a process analytical technology tool [21]. In addition, obtaining the Raman spectra at each position of the formulation provides information on the spatial distribution of components, as well as their physical states, which are valuable for troubleshooting manufacturing processes [22], monitoring blend homogeneity [23], revealing the internal structure [24], and understanding the physical attributes [25] of the products.

The overall aim of this study was to evaluate the feasibility, methodology, and possible future applications of Raman spectroscopy for the quality evaluation of DMNAs. Herein, we report the chemical imaging of a drug in sodium hyaluronate (HA)–based films and drug-loaded DMNAs using Raman microscopy. We selected diclofenac sodium (DCF) as a model compound. As a widely used non-steroidal anti-inflammatory drug (NSAID), DCF can help compare the advantages and limitations of transdermal systems, including safety and ability to penetrate through the skin and achieve therapeutic concentrations in deep subcutaneous tissues [26].

**Materials and methods**

**Materials**

Sodium hyaluronate (HA) (FCH-SU, lot: HGV016900, average molecular weight (MW): 50,000–110,000 Da) was purchased from Kikkoman Biochemifa Company (Tokyo, Japan). Diclofenac sodium (DCF) was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Polydimethylsiloxane (PDMS, DowSil Silpot 184) was purchased from Dow Corning (Midland, MI, USA). Aluminum microneedle mold masters, fabricated via micro-milling, were purchased from Tokai Azumi Techno (Mie, Japan). All other chemicals were purchased commercially as reagent-grade products. Water was deionized and purified using a Milli-Q® TOC purification system (Millipore, Bedford, MA, USA).

**Preparation of HA films**

We used HA as a base polymer because it is a component of the skin tissue with a hydrophilic nature; thus, it may be biocompatible with the skin and safe for DMNA insertion [6, 11, 27]. DCF was dissolved in distilled water, and then, 10% (w/w) HA was added and uniformly mixed. Each solution was poured into a silicone rubber mold consisting of a circular area with a diameter of 14 mm. To allow the solution to spread evenly, the mold was placed on a flat surface and dried in a desiccator at room temperature for 24 h.

**DMNA fabrication**

DMNA was fabricated using micromolding technology with HA as the base polymer. First, the microneedle mold masters were prepared with the following geometry: 316 conical microneedles, 140-µm base diameter, 40-µm tip radius, and 0.80-µm height. Next, inverse replication of the master structures was reproduced using PDMS. The DCF-containing HA solution was prepared in the same manner as the HA films. The aqueous solution was poured into PDMS micromolds. The molds were then centrifuged at 3500 rpm for 15 min at room temperature to allow the solution to fill the holes. After that, the molds were kept for 48 h at 35 °C to dry the needles. The DMNA was obtained by separating them from the molds and cutting them into circles of 14 mm in diameter using a punch. The morphology of DMNA was investigated using a Keyence VHX-5000 digital microscope (Keyence, Osaka, Japan). Supplementary Fig. 1 shows a diagrammatic representation of the fabrication method.

**Quantification of the DCF content**

The DCF content was determined using HPLC. HA films were dissolved in 10 ml of distilled water at room temperature using a SR-2DW shaker (TAITEC, Saitama, Japan). The amount of the drug in DMNA needle was assayed as described previously [11]. Needles were carefully removed from the baseplates, under a magnifying lens, using a razor. Subsequently, the needles were dissolved in 1.0 ml distilled...
water at room temperature by vortexing. The sample solution was analyzed using HPLC by referring to the Japanese Pharmacopoeia 18 diclofenac sodium suppositories. Briefly, an LC-20A HPLC system (Shimadzu, Kyoto, Japan) with an Inertsil ODS-3 column (4.0 x 150 mm, 5-µm particle size) was used. The mobile phase comprised 0.1 M sodium acetate trihydrate and methanol (40:60, v/v). The flow rate and column temperature were 1.1 ml/min and 25 °C, respectively, and the injection volume was 50 µl. HPLC was performed at a detection wavelength of 254 nm. The concentration of DCF in the sample solution was determined using a standard curve based on the known concentration of DCF. Data were reported as drug content (in mg) per film and drug content (in mg) in DMNA needles per array (mean ± SD, n = 2–3).

**PXRD analysis**

PXRD analysis of the HA films and reference materials was performed using a powder X-ray diffractometer (MiniFlex 600, Rigaku, Tokyo, Japan). The sample was spread on a non-reflective plate. The diffraction angle, scan rate, voltage, and current of the generator were set to 2.0 to 40.0°, 4.0°/min, 40 kV, and 15 mA, respectively.

**Raman fiber probe analysis**

A Raman fiber probe was used to detect the Raman spectra of the samples. An iHR320 spectrometer system (Horiba, Kyoto, Japan) with a CCD detector (Syncerity Scientific Cameras, Horiba) was used. A 100-mW, 785-nm excitation laser was connected to the incidence fiber, while the Raman signal was collected through a 0.5-inch diameter fiber ball probe (MarqMetrix, Seattle, WA, USA). Raman spectra were collected four times at an acquisition time of 90 s by directly touching the samples with the probe head. The relative Raman intensity ratio of the DCF characteristic peak at 1579 cm⁻¹ to the HA characteristic peak at 1376 cm⁻¹ was determined as an internal control using the equation below:

\[
\text{Relative Raman intensity ratio of DCF} = \frac{\text{DCF characteristic peak intensity at } 1579 \text{ cm}^{-1}}{\text{HA characteristic peak intensity at } 1376 \text{ cm}^{-1}}
\]

The relative Raman intensity ratio of DCF = 2.4%.

**Confocal Raman microscopy**

Confocal Raman microscopy (LabRAM HR Evolution, Horiba) was used to obtain the Raman spectra of the samples. The samples were excited with a 785-nm laser using a 20 x microscope objective. In the case of HA films, a 50-µm step size was used with an exposure time of 4 s, scanning through a 2000-µm square area (total 1681 points). In the case of DMNA, a 10-µm step size was used with an exposure time of 4 s, scanning through a 180 x 900 µm square area (total 1729 points). Raman signatures of the samples were acquired in the fingerprint region (800 – 2100 cm⁻¹). LabSpec 6.0 software was used for spectral collection, spectral preprocessing, and data set processing. The obtained spectra were preprocessed to remove the influence of fluorescence, and then, the spectra were used to generate chemical images using the classical least-squares (CLS) algorithm or each component characteristic peak intensity.

**Statistical analysis**

The results were presented as the mean ± standard deviation of the mean. The slope and intercept of the curves were estimated via least-squares regression using GraphPad Prism software (ver. 8; GraphPad, Inc. San Diego, CA, USA).

**Results and discussion**

**Preparation and characterization of DCF-loaded HA films**

In this study, we first prepared a drug-polymer film as a simple model. MW and concentration of HA directly affect the solution viscosity; therefore, we chose a relatively low MW (50,000–110,000 Da) and 10% (w/w) concentration for the ease of handling. The amount of DCF used to prepare the DCF-loaded HA films at ratios of 1:5, 1:10, 1:20, and 1:40 were approximately 16.7, 9.1, 4.8, and 2.4% by weight, respectively. Figure 1 shows the digital microscopic images of the DCF-loaded HA films. At the DCF concentration range of 4.8 to 16.7% (w/w), uniform surface crystallization was observed, and a concentration-dependent increase in surface crystallization was observed. However, at a DCF concentration of 2.4%, visible DCF crystals were observed. In contrast to the crystallization observed in the films with a concentration greater than 4.8%, where fine crystals grew from a significant number of nuclei, the crystals in the 2.4% film grew from a limited number of nuclei. HPLC for the quantification of DCF content in HA films (2.4, 4.8, 9.1, and 16.7% (w/w)) showed 0.42 ± 0.05, 0.98 ± 0.12, 1.98 ± 0.12, and 3.55 ± 0.29 mg/film, respectively. Linearity was evaluated by assessing the slope, intercept, and coefficient of determination (r²) for the linear fit of the quantitative value (mg/film) as a function of the theoretical drug loading ratio (%): y = 0.218 x - 0.068, r² = 0.999. These results indicated that linearity was observed at certain mixing ratios of DCF in HA films.
To investigate the crystallinity of DCF after loading into the HA polymer matrices, PXRD analysis was performed (Fig. 2). XRD patterns of the reference reagent DCF powder were in agreement with those reported previously [28]. The non-drug-loaded HA film showed a narrow-amorphous pattern. In contrast, the XRD patterns of the DCF-loaded HA films showed multiple sharp peaks, indicating crystalline DCF in the films. Different peak positions of XRD patterns in the DCF-loaded HA films suggested different physical and/or chemical states of DCF in the solids. It has been reported that DCF acid, DCF, and their hydrates crystallize on several crystal forms depending on the preparation method. For example, Muangsin et al. reported that the crystal structure obtained for DCF recrystallized from the matrix is DCF pentahydrate [29]. The available XRD patterns of DCF, DCF pentahydrate [28], and two polymorphic forms (monoclinic P2₁/c and monoclinic C2/c) of DCF acid [30], however, did not match those of the DCF-loaded HA films in our study.

Standard Raman spectra of each component

The first step was to determine the reference Raman spectra of DCF and HA. The Raman spectra of DCF and HA corresponded to those reported previously (Fig. 3) [31, 32]. In the Raman spectra of DCF, the characteristic peak at 1579 cm⁻¹ was assigned to the asymmetric stretching vibration of the carboxylate. In the Raman spectra of HA, the characteristic peak at 1376 cm⁻¹ was assigned to the C–H bend vibration. The characteristic and non-overlapping Raman peaks enabled the identification of HA and DCF amount required for the following experiments.

Evaluation of DCF-loaded HA films using a Raman fiber probe

Next, we examined the relationship between the DCF content and Raman spectra. Figure 4A shows the Raman spectra of HA films loaded with various amounts of DCF, obtained using a Raman fiber probe. We utilized the spherical lens Raman probe...
because it allowed highly sensitive and reproducible measurements by touching the lens directly to the sample. The relationship between the amount of DCF and the relative Raman intensity ratio of the DCF characteristic peak at 1579 cm$^{-1}$ to the HA characteristic peak at 1376 cm$^{-1}$ (Fig. 4B) indicated a linear increase in the peak intensity with the theoretical drug loading ratio (%): $y = 0.1013 x - 0.0490$. These results suggested that Raman spectroscopy is feasible for the identification and quantification of DCF in HA films despite possible differences in DCF spectra depending on the physical state.

The results obtained for the films loaded with 2.4% DCF are not included in Fig. 4 because of the large variation in the peak intensity. Reproducible Raman spectra were obtained from 4.8 to 16.7% (w/w) DCF, but not at 2.4% (w/w) (data not shown). It is possible that the visually observable large crystal appearing on the surface of the lower dose DCF films induces a large variation in the intensity of the DCF-specific Raman peak depending on the measurement area. This technical problem can be solved by obtaining data from a wider area [33].

Fig. 3 Raman spectra of reference materials. (A) HA and (B) DCF. Asterisk and double asterisk indicate the HA characteristic peak at 1376 cm$^{-1}$ and DCF characteristic peak at 1579 cm$^{-1}$.
Chemical imaging analysis of DCF-loaded HA films by confocal Raman microscopy

To examine the spatial distribution patterns of DCF in the films, two-dimensional Raman imaging analysis was performed using confocal Raman microscopy. Figure 5A–D, E–H show optical and Raman images of DCF-loaded films, respectively, obtained by CLS. The films containing intermediate to high amounts of DCF (Fig. 5E–G) showed an increasing number of small DCF crystals on their surface with increasing DCF content. In contrast, the films containing lower amounts of DCF (Fig. 5H) showed an uneven distribution of DCF, potentially due to large or agglomerated particles, which is consistent with the optical image and Raman fiber probe results. The absence of DCF in the HA films resulted in the absence of a characteristic DCF peak (1579 cm\(^{-1}\)) in Raman spectroscopy (data not shown). These results demonstrate the applicability of Raman imaging analysis to characterize the spatial distribution of APIs in HA-based films.

Fabrication and pharmaceutical characterization of DCF-loaded DMNAs

We fabricated HA-based DMNAs loaded with varying amounts of DCF using micromolding technology to evaluate the applicability of Raman imaging and assess the spatial distribution of the API in DMNA needles. Figure 6A–C shows a micrograph of the non-drug-loaded DMNA using a digital microscope. The resulting tapered cone DMNA was uniform in size (approximately 800 μm in length) with sharp tips, which allows SC penetration without apparent pain [34].

We prepared four different concentrations (2.4, 4.8, 9.1, and 16.7% (w/w)) of DCF-loaded DMNA in the same way. However, the addition of DCF induced bending of some needles during the process in a concentration-dependent manner (data not shown), which suggested a compromise in the strength of DMNA needles, as reported by Donnelly et al. [35]. For this purpose, DMNAs prepared using the same procedure were used for their characterization. HPLC analysis indicated that the amount of the drug (2.4, 4.8, 9.1, and 16.7% (w/w)) in DMNA needles was 0.0074 ± 0.0026, 0.0516 ± 0.0150, 0.1789 ± 0.0554, and 0.3591 ± 0.0174 mg/array DCF, respectively.

Chemical imaging analysis of DCF-loaded DMNA using confocal Raman microscopy

Finally, we performed Raman imaging analysis to evaluate the spatial distribution of DCF in DMNAs. To locate a single needle in the lateral direction (Y-axis), we attached DMNA to a stationary 90° edge with double-sided adhesive tape (Fig. 7). The optical and Raman images of DMNAs with and without 16.7% DCF (w/w) were successfully collected over a large area enough to hold a single needle. The univariate Raman image of the characteristic HA peak (1376 cm\(^{-1}\)) intensity (Fig. 7B) showed a broad distribution of HA on

![Figure 5](image_url)  
**Fig. 5** Raman spectroscopy mapping of DCF-loaded HA film, optical images (A–D), and Raman images generated by CLS (E–H). A, E 16.7%, B, F 9.1%, C, G 4.8%, D, H 2.4% DCF (w/w). Green and blue colors represent DCF and HA, respectively. The scale bars are 200 μm
the surface of the needle prepared without DCF. In contrast, HA was scattered on the surface of the DCF-loaded DMNA needle (Fig. 7F), suggesting that another component existed along with HA. The univariate Raman image of the DCF characteristic peak (1579 cm\(^{-1}\)) intensity (Fig. 7G) showed the distribution of DCF on the surface of the DCF-loaded DMNA needle. The absence of added DCF resulted in a negligible Raman peak intensity (Fig. 7C). Overlapped figures (Fig. 7D and H) clearly indicated the distribution of DCF on the surface of HA-based needles.

The results indicated the applicability of the Raman imaging to assess drug distribution in DMNAs, which is relevant to ensure the safety and efficacy of formulations. In the conventional method, the blend of drugs and polymers is mixed with fluorescent tracer or dye, and the filling position of the drug is confirmed using fluorescent tracer and dye as an index [17, 18]. However, these methods require the fluorescent tracer and dye to be encapsulated in needles in addition to drugs, which is considered useful for basic research, but unsuitable for considering clinical applications. In addition, these methods determine the location of fluorescence and dye, but it is not clear whether they reflect the exact distribution of drugs. Therefore, the advantage of Raman method is visualizing the distribution of the drug itself without any tracer. The availability of the drug position in the needles is the apparent advantage of Raman imaging over other methods (e.g., use of fluorescent tracer). Combinations of Raman imaging with certain quantitative methods (e.g., HPLC) would provide valuable information for the development and production of DMNA products. This method may also be valuable for developing complex DMNA formulations that control drug distribution in various ways (e.g., use

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**Fig. 6** The optical image of non-drug-loaded dissolving microneedle array. A \(\times 30\) magnification viewed just above, B \(\times 150\) magnification viewed from 45°, C \(\times 150\) magnification viewed from 90°

**Fig. 7** Raman spectroscopy mapping of DCF-loaded DMNA, non-drug-loaded DMNA (A–D), 16.7% DCF (w/w)-loaded DMNA (E–H), optical images (A, E), Raman images generated by HA characteristic peak intensity at 1376 cm\(^{-1}\) (blue; B, F), DCF characteristic peak intensity at 1579 cm\(^{-1}\) (green; C, G), and merge (D, H). The scale bars are 50 µm
of drug-loaded microparticles and drug loading to the tip of a microneedle) [36].

In this first study, we aimed to discern the two-dimensional distribution of the drug in DMNAs. However, if possible, it would be more useful to discern the three-dimensional distribution of drug without samples destruction. Several studies have reported that confocal Raman imaging allowed visualization of the distribution of components of semi-solid dosage forms in three dimensions [37]. Similarly, it is thought that the three-dimensional distribution can be clarified for DMNA needles because, unlike tablets, they are translucent and the excitation laser can enter the inside. Future studies will focus on visualizing the three-dimensional distribution of drugs within a needle. Obtaining appropriate spectral data and assessing them is a major challenge for the anticipated broad application of this method. The complex shape of the small needles makes precise analysis challenging owing to difficulties in setting the appropriate focus in the measurement area, which requires an autofocusing system. Improving the data processing method to obtain the physical properties of the components (e.g., crystallinity, crystal form) is another challenge, which would increase the available information for the analysis.

Conclusion

Our results suggest that Raman spectroscopy is a useful tool for determining the spatial distribution of drugs in DMNAs. Availability of the distribution information in the formulation would be an apparent advantage over other methods (e.g., use of fluorescent tracer, HPLC), which is valuable for the development and manufacturing (e.g., troubleshooting) of DMNA products.

Supplementary Information

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Author contribution

D.A. designed the study, performed experiments, and wrote the manuscript. T.M. and T.K. assisted with writing the manuscript. E.Y. and K.I. directed the research and assisted in writing and editing the manuscript. All the authors have provided comments on the manuscript.

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Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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