Mechanistic modeling-guided optimization of microneedle-based skin patch for rapid transdermal delivery of naloxone for opioid overdose treatment

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Abstract
Naloxone, an FDA-approved opioid inhibitor, used to reverse opioid overdose complications has up till date faced challenges associated with its delivery. Limitations include the use of invasive delivery forms and the need for frequent redosing due to its short half-life. The goal of the current study was to design a transdermal rapidly dissolving polymeric microneedle (MN) patch with delivery and pharmacokinetic properties comparable to that seen with the commercially available NAL products, eliminating their delivery limitations. Patches of varying dimensions (500 µm; 100 array, 800 µm; 100 array, and 600 µm; 225 array) were fabricated to evaluate the effect of increasing MN length and density (no. of needles/unit area) on drug release. Drug dose in each of these patches was 17.89 ± 0.23 mg, 17.2 ± 0.77 mg, and 17.8 ± 1.01 mg, respectively. Furthermore, the insertion efficiency of each of the MN patches was 94 ± 4.8%, 90.6 ± 1.69%, and 96 ± 1.29%, respectively. Compared to passive permeation, a reduced lag time of about 5–15 min was observed with a significant drug flux of 15.09 ± 7.68 g/µl/cm²/h seen in the first 1 h (p < 0.05) with the array of 100 needles (500 µm long). Over 24 h, a four and ten-fold increase in permeation was seen with the longer length and larger density MN patch, respectively, when compared to the 500 µm (100 array) patch. Model simulations and analyses revealed the significance of needle base diameter and needle count in improving systemic pharmacokinetics of NAL.

Keywords Microneedles · Transdermal delivery · Naloxone · Opioid · Polyvinylpyrrolidone (PVP)

Abbreviations

CLSM Confocal laser scanning microscopy
FDA Food and Drug Administration
GSA Global sensitivity analysis
HIV/AIDS Human immune deficiency syndrome/Acquired immune deficiency syndrome
HPLC High performance liquid chromatography
NAL Naloxone
NIDA National Institute on Drug Abuse
MN Microneedle
IE Insertion efficiency
IN Intranasal
IM Intramuscular
IV Intravenous
LSA Local sensitivity analysis
GSA Global sensitivity analysis
PBS Phosphate buffered saline
PDMS Polydimethylsiloxane
PK Pharmacokinetics
PVP Polyvinylpyrrolidone
SC Stratum corneum
TFA Trifluoroacetic acid

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Introduction

Opioid use disorder is an age-long societal menace affecting over 16 million people in the world [1]. Death due to overdose is quite prevalent with opioid abuse [2–5]. At the global level, over 100,000 opioid overdose-related deaths are reported annually with 56,064 deaths reported in the USA alone in 2020 by the CDC’s National Center for Health Statistics [6, 7]. A breakthrough therapeutic for management of opioid overdose emergencies is naloxone (NAL), approved by the FDA in 1971 and commonly sold under the brand name, Narcan [8–10]. It is currently administered via the intravenous (IV), intramuscular (IM), subcutaneous (SC), and intranasal (IN) route. With IV delivery, even though a desired rapid response is seen within 2 min of drug administration [11], the rapid systemic clearance of the drug and hence its short half-life of about 30–81 min oftentimes warrant repeat dosing which is usually recommended to be given every 2–3 min depending on individual recovery seen and severity of overdose [10, 12, 13]. Typically, [10] from a patient perspective, this is deemed not an ultimately preferred option considering the invasive nature of IV delivery, causing needless pain and discomfort through the course of patient stabilization. Furthermore, safety issues relating to inflammation and damage to blood vessels from prolonged drug delivery via the IV route are prevalent [14, 15]. It is also a common challenge to have difficulty accessing the vasculature for IV injections since most abusers have their veins damaged from chronic self-drug administration [11, 13]. The need for trained medical intervention at all times seems not so appropriate as well for a drug set for use in emergencies; preferably, this should be available in a form ready for use by lay individuals or first responders in overdose situations [4, 11]. Furthermore, caregivers are at risk of deadly communicable infections such as HIV/AIDS or hepatitis via accidental needle stick injuries [16].

Oral delivery of NAL on the other hand is impractical due to its significant hepatic metabolism and consequently poor oral bioavailability of about 2% [17]. Efforts to develop alternative non-invasive delivery options brought in its wake the approval of NAL intranasal (IN) spray [4, 11]. This is however not devoid of limitations as well and some of these include the inability to use route due to injuries to the nasal mucosa common in drug addicts from drug snorting [13, 18]. Response variation among users due to nasal congestion, epistaxis, and infection is also a challenge [11, 13]. Efforts to develop transdermal formulations for NAL has been previously reported, justification for this being its non-invasiveness and capability for sustained release and minimized side effects. Though, these attempts have demonstrated the feasibility of successfully delivering NAL via the transdermal route, a critical limitation that could not be surpassed was the lag time to delivery which ranged from 30 min to 1 h. Puri et. al demonstrated the prospect of using solid MNs to shorten this and to improve flux through skin. However, for clinical translatable, there is a need for an improvised delivery system. Limitations identified with the use of solid MNs as highlighted in numerous reviews [19–24] warrant devising more practical MN formulation options, and hence, NAL loaded dissolvable MN patch was designed in this study.

In the present study, PVP-based NAL MNs were fabricated. Optimal fabrication processes required for the formulation of consistently efficient NAL MNs in terms of penetration and permeation efficiency through skin was determined. To our knowledge, this is so far the first study to fabricate MN skin patches for NAL transdermal delivery. Herein, the optimized microfabrication process for NAL PVP MN, its permeation profile, MN optimization considerations, and characterization reports on the fabricated MNs are reported.

Of note, we developed a mechanistic mathematical model to simulate the dissolution of conical MNs and study drug release from dissolvable MN-based patches. For our work, we adapted previously published mathematical models that described the dissolution process of MNs to predict the delivery of drugs into plasma. Kim et.al developed a model for a conical microneedle from which a surrounding shell dissolved at every time point to quantify the dissolved microneedle volume kinetics. Similarly, Zoudani and Soltani et al. implemented a mathematical model to understand the dissolution process of conical microneedles in porous medium, using the same shell dissolving process as above, and incorporated an array of hemispherical convexities loaded with drug on the tip of the microneedle to decrease the drug delivery time [25, 26]. In the current study, we assumed a modified dissolution process such that instead of the needles shrinking in thickness due to dissolution, we assumed shrinkage of length as they dissolve, to better reflect the observations in the literature [19, 27]. Furthermore, based on our previous works [13], by integrating to a two-compartment PK model, our model can predict the clinical PK of NAL. Through comprehensive parameter analyses, the model was thus used to optimize the design of patches to improve drug release characteristics and enhance plasma bioavailability of NAL to match the clinical performance of FDA-approved devices for NAL delivery.

Materials

NAL hydrochloride was purchased from Thermo Fisher Scientific (Ward Hill, MA, USA). Polydimethylsiloxane (PDMS) molds were obtained from Micropoint Technologies.
Trifluoroacetic acid (TFA), potassium monophosphate, 10X phosphate buffered saline, pH 7.4 (PBS), PVP (MW = 40,000), and silver wire (0.5 mm diameter, 99.99%) were purchased from Fisher Scientific (Fair lawn, NJ, USA). Methanol was purchased from Concord Technology (Beichen, Tianjin, China). Silver/silver chloride electrodes (2 mm × 4 mm) were obtained from A-M systems (Sequim, WA, USA). Flourescein isothiocyanate was bought from Sigma Aldrich (St. Louis, MO, USA). Methylene blue was obtained from Electron Microscopy Sciences (Hatfield, PA, USA). Porcine ears were procured from Animal Technologies (Tyler, Texas, USA).

Methods

Solubility study

The solubility of NAL in 7.5% PVP and 10 mM PBS, pH 7.4 (1X PBS) was determined. For all, an excess amount of NAL was added to 200 µL of these solvents and left for shaking (Orbi-Shaker BT302, Cambridge Scientific, Boston, MA) at room temperature for 24 h. The solution was then centrifuged (05–090-128 Mini Centrifuge, Fisher Scientific, Fair Lawn, NJ) and the supernatant was diluted 10,000 times with 1X PBS and analyzed using reverse phase high performance liquid chromatography (RP-HPLC, n = 3) [13].

Characterization of NAL MNs

Bright field imaging

Imaging was done using the Omax brightfield microscope (OMAX MD82EZ10, China). A sample holder was manually formed in the lab to fix MNs on the microscope stub. This was done using double-sided tapes, microscope glass slides, and a parafilm bed to hold the MNs at an angle suitable for viewing it sideways. This was used to evaluate MNs for crystals, qualitative and quantitative assessments including the evaluation of needles for surface morphology, needle tip integrity, uniformity, and dissolution post-insertion.

Fabrication of NAL MNs

NAL-loaded MNs with varying dimensions were prepared with details shown in Table 1. This was done using the mold casting technique with PDMS molds. A known amount of drug was dissolved in 7.5% solution of PVP in distilled water to prepare casting solutions of varying drug strength ranging from 50 through 150 mg/ml of NAL in the preformed PVP solution. The drug solution (170 µL) was poured in the molds and vacuumed at room temperature and a constant pressure of about −0.6 psi for 5 min with iterative degassing steps. Following the full elimination of air bubbles in mold filled solutions, 130 µL of drug in 7.5% PVP solution was added. Full degassing was further ensured and vacuum with same condition as starting steps was applied for at least 1 h and left for drying. The mold was filled up to its brim after 24 h with 130 µL of drug in 7.5% PVP solution to strengthen and thicken the base of the needle. The needles were left in the desiccator for drying at room temperature for two more days. Following this, needles were gently separated from the mold and transferred onto a 3 M adhesive tape backing for application on skin. The mold casting technique is illustrated in Fig. 1.

Table 1 Dimensions of fabricated NAL MN patches

| Code for fabricated MNs | Array size (# of needles) | Needle height (µm) | Needle base (µm) | Needle pitch (µm) | Patch size (mm × mm) |
|-------------------------|---------------------------|-------------------|-----------------|------------------|--------------------|
| P1                      | 100                       | 500               | 200             | 500              | 8 × 8              |
| P2                      | 100                       | 800               | 200             | 500              | 8 × 8              |
| P3                      | 225                       | 600               | 200             | 500              | 8 × 8              |

Fig. 1 Schematic illustration of the mold casting procedure followed for fabrication of NAL PVP-based MNs

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for 2 min. All these were determined by running across the entire needle array using the x 40 magnification lens. Images were taken using the Toup view imaging software. Calibration was done with a 1 mm rule to measure MN length, tip to tip distance, tip to base, and base to base distance (n = 6). Results are presented as mean ± SE and compared to the vendors specifications.

Dye binding and skin insertion efficiency

Skin pieces were obtained and thawed, dabbed with Kimwipes to remove moisture from thawing. MNs were applied using thumb insertion force for 2 min. Thereafter, a drop of methylene blue dye was applied for about 20 min. Dye on the skin was wiped off with Kimwipes and the skin was gently cleaned with alcohol swabs. Evaluation of the stained skin for pore formation was done under the microscope [12]. Furthermore, skin insertion efficiency was determined by counting the number of pores per porated skin (n = 3–4) and reporting as a percentage based on the total number of needles on the array mold provided by the vendor ± SE.

Confocal laser scanning microscopy

The depth of the microchannels created by MNs was analyzed using confocal laser scanning microscopy (CLSM). Fluorescein isothiocyanate (0.35%, 200 µL) was applied to the skin after treatment with MNs for 1 min. Excess dye was removed with Kimwipes. The MN-treated skin samples were placed on a glass slide without distortion or fixation artifacts. The slides were observed using a computerized Leica TCS SPE II confocal microscope (Leica Microsystems, Heerbrugg, CH9435 Switzerland) with 10X objective at an excitation wavelength of 488 nm. Leica Application Suite X 3.5.5.19976 fluorescence software was used to process the images. The depth of the microchannels created and pattern of distribution of fluorescein in the microchannels were studied using X–Z sectioning [13].

Drug content

Fabricated MN arrays (n = 4) were separated gently, dissolved in 20 ml of 1X PBS and vortexed. The solutions were then diluted 100 times with 1X PBS and HPLC was used to quantitate the drug content in each diluted solution. The quantity of drug in each sample ± SE was determined by the standard curve method.

In vitro permeation study

Porcine ear skin was trimmed and dermatomed, cut into appropriate pieces (thickness ranging between 0.6 and 0.9 mm), and stored in a freezer at −20 °C. Skin pieces were removed on the day of the study and thawed in 1X PBS solution (pH 7.4) at 37 °C for 2–3 min. Receptor for Franz cell diffusion chambers was filled up with 5 ml of 1X PBS. Prepared skin pieces were mounted in between the receptor and donor. The donor chambers were filled with 300 µL of 1X PBS. After about 15 min of equilibration, skin integrity was determined by measuring skin resistance. Silver/silver chloride electrodes, an arbitrary 2 channel waveform generator (RIGOL DG822, 25 MHZ, TEquipment, NJ, USA), and a digital micrometer (RIGOL DM 3068 61/2 digit, TEquipment, Long Branch, NJ, USA) were used for this. The load resistor R_L (silver chloride, 100 kΩ) was dipped in the donor solution. The value on the multimeter was recorded. Skin pieces with display values ranging from 15 to 50 mV were selected for the studies. Resistance was then calculated using the formula:

\[ R_S = \frac{V_S \cdot R_L}{V_O - V_S} \]  

where V_S is the voltage drop across the entire circuit, R_L is load resistance, and V_O is voltage drop across skin. V_O and R_L were 100 mV and 100 kΩ, respectively. Care was taken to ensure that the resistances of the skin pieces within the groups all through studies were comparable. After measuring the resistance, the donor solution was removed, and dried with Kimwipes. Skin temperature was measured using IR thermometer ensuring that they were all about 32 °C. The MN patches of varying dimensions as detailed in Table 1 were adhered onto the skin using the adhesive backing and thumb force for 2 min was applied for microporation on the selected skin pieces. The skin pieces with the patches adhering to them were then mounted on the Franz cells and left for 24 h. The control group assessed the passive permeation of NAL across intact dermatomed porcine ear skin after applying 430 µL of 50 mg/ml NAL in 7.5% PVP solution in the donor chamber (n = 5). Receptor samples (300 µL) were taken at 0 h, 0.083 h, 0.25 h, 0.5 h, 0.75 h, 1 h, 2 h, 4 h, 5 h, 6 h, 8 h, 22 h, and 24 h, into sample vials and filtered. Equal volume of fresh buffer solution was replaced in receptor chamber of Franz cells. Samples were then analyzed for drug amount using HPLC. The result for each test group was reported as mean ± SE. The cumulative amount of NAL permeated through skin per unit area was plotted as a function of time. The slope of the linear portion of the plots over the first hour and over 24 h was used to calculate the flux of NAL from the patch [13, 28].

Quantitative analysis of NAL

HPLC analysis for NAL was done using the HPLC Waters Alliance 2695 separation module (Cambridge Scientific, MA, USA). The validated method used was an isocratic elution that involved the use of Kinetex® biphenyl 100 Å
Mathematical modeling

Mathematical model development

We adapted the Nernst-Brunner equation [26] (Eq. (2)) to model the dissolution kinetics of MN patches and quantify the release of NAL into the Franz cell.

\[
\frac{dM(t)}{dt} = k_D \cdot (c - c(t)) \cdot A(t) \cdot M(0) = 0 \tag{2}
\]

Here, \(M(t)\) is the dissolved mass of the patch; \(k_D\) is the dissolution rate constant of the matrix polymer, which is equal to the ratio of diffusion coefficient \((D)\) of the polymer to the thickness \((c)\) of the unstirred layer (i.e., \(k_D = \frac{D}{c}\)); \(A(t)\) is the available surface area of the patch for dissolution; \(c\) is the solubility of the polymer, and \(c(t)\) is the concentration of the dissolved polymer in bulk solution.

As a simplification, we applied the above equation to a single repeating unit of the patch, defined by a single microneedle and the supporting piece of baseplate (or backing) and thus obtained the cumulative mass kinetics of the entire patch by multiplying over \(N\) repeating units (Fig. 2a). Note that as a further simplification, we assumed \(c(t) = 0\), suggesting that polymer concentration in the skin is effectively zero due to its rapid transport into the Franz cell solution in vitro, or analogously into the systemic circulation in an in vivo setting. Therefore, we obtained:

\[
\frac{dM(t)}{dt} = k_D \cdot c_s \cdot (A_{MN}(t) + A_B(t)) \cdot M(0) = 0 \tag{3}
\]

where \(A_{MN}(t)\) and \(A_B(t)\) are the available surface areas of a single microneedle and its associated baseplate, respectively.

To estimate the available MN surface area \((A_{MN}(t))\), we assumed that the MN is shaped like a cone that dissolves starting from its tip and ending at its base (Fig. 2b), as observed in the literature [29, 30]. Therefore, at any time point \(t\), the MN can be viewed as a truncated cone with surface area available for dissolution given by:

\[
A_{MN}(t) = \pi \cdot (r + r_c(t)) \cdot \sqrt{(h - \Delta z(t))^2 + (r - r_c(t))^2} + \pi \cdot r_c(t)^2 \tag{4}
\]

where \(r\) and \(r_c(t)\) are the large and small radius of the truncated cone, respectively; \(h\) is the initial height of the intact MN; and \(\Delta z(t)\) is the dissolved height of the MN.

The half angle at the apex of the cone \((\theta)\) is related to the radius and the height of the intact cone as \(\tan \theta = \frac{r}{h}\), and by similarity of triangles, we can also relate it to the parameters of the truncated cone, such that \(\tan \theta = \frac{r_c(t)}{h - \Delta z(t)}\) (Fig. 2c). With this relation, we can rewrite Eq. (4) in terms of the MN parameter \(\theta\) as:

\[
A_{MN}(t) = \pi \cdot \frac{\tan \theta}{\cos \theta} \cdot (h^2 - \Delta z(t)^2) + \pi \cdot \tan^2 \theta \cdot \Delta z(t)^2 \tag{5}
\]

Furthermore, since in the Nernst-Brunner equation we are modeling the dissolved mass \(M(t)\) of the MN, therefore, to retain only one variable in the model, in Eq. (5) we rewrite the dissolved height of the cone \(\Delta z(t)\) in terms of the corresponding dissolved mass \(M(t)\). Given the volume of a cone being \(\pi \cdot r^2 \cdot \frac{h}{3}\), \(\rho\) as the density of the MN, and \(\tan \theta = \frac{r_c(t)}{h - \Delta z(t)}\), we obtain the mass of the dissolved cone:

\[
M(t) = \rho \cdot \pi \cdot \tan^2 \theta \cdot \left(\frac{\Delta z(t)^3}{3}\right) \tag{6}
\]

Solving for \(\Delta z(t)\) in the above expression and substituting its value in Eq. (5), we obtain:

\[
A_{MN}(t) = \pi \cdot \frac{\tan \theta}{\cos \theta} \cdot \left(h^2 - \sqrt{\left(\frac{3 \cdot M(t)}{\rho \cdot \pi \cdot \tan^2 \theta}\right)^2}\right) + \pi \cdot \tan^2 \theta \cdot \sqrt{\left(\frac{3 \cdot M(t)}{\rho \cdot \pi \cdot \tan^2 \theta}\right)^2} \tag{7}
\]

Furthermore, the surface area of dissolution for the associated baseplate of the MN is \(A_B(t) = \frac{S}{N}\), where \(S\) is the total surface area of the patch and \(N\) is the number of MNs (or repeating units) in the patch. Substituting this expression and Eq. (7) in Eq. (3), we obtain:

\[
\frac{dM(t)}{dt} = k_D \cdot c_s \cdot \left\{ \pi \cdot \tan \theta \cdot \left[ h^2 - \sqrt{\left(\frac{3 \cdot M(t)}{\rho \cdot \pi \cdot \tan^2 \theta}\right)^2}\right] + \tan \theta \cdot \sqrt{\left(\frac{3 \cdot M(t)}{\rho \cdot \pi \cdot \tan^2 \theta}\right)^2} \right\} 1_{h \geq \Delta z(t)}(t) + \frac{S}{N} \cdot \frac{1}{1 \geq h \geq M(t)} \cdot M(0) = 0 \tag{8}
\]

where, \(1_{h \geq \Delta z(t)}(t) = \begin{cases} 1, & h \geq \Delta z(t) \\ 0, & h < \Delta z(t) \end{cases}\)
\[ \Delta z(t) = \sqrt{\frac{3 \cdot M(t)}{\rho \cdot \pi \cdot \tan^2 \theta}} \]

and \( \dfrac{1}{\Delta z(t)} \geq M(t) \) = \( \begin{cases} 1, & \text{if } \frac{\text{Dose}}{N} \geq M(t) \\ 0, & \text{if } \frac{\text{Dose}}{N} < M(t) \end{cases} \)

Note that we did not assume any time delay in the initiation of dissolution of baseplate, i.e., the MNs and baseplate begin to dissolve simultaneously upon administration.

By solving the above equation and multiplying with the \( N \) repeating units of the patch, we obtained the total cumulative mass (\( M(t) \)) of the dissolved patch. From this, we estimated the total cumulative mass of NAL (\( M_{\text{NAL}}(t) \)) released by the patch into the Franz cell by multiplying with the drug loading fraction \( \beta \) of the patch:

\[ M_{\text{NAL}}(t) = \beta \cdot N \cdot M(t) \]  

Equation (9) was numerically solved in MATLAB using the built-in ODE solver \texttt{ode45}. Non-linear least squares regression of the model solution to in vitro release kinetics data for NAL was performed to estimate the unknown model parameter \( k_D \), using the built-in MATLAB function \texttt{lsqcurvefit}. The remaining model parameters were known a priori (Table 2). Furthermore, from the numerical solution of the model, the time-dependent flux of NAL (\( J_{\text{NAL}}(t) \)) was estimated as \( J_{\text{NAL}}(t) = \frac{M_{\text{NAL}}(t+\Delta t)-M_{\text{NAL}}(t)}{\Delta t} \), to which spline interpolation was performed to obtain a time-dependent function of flux for application in pharmacokinetic analysis.

**Pharmacokinetic modeling**

To predict the clinical pharmacokinetics of MN patch-based drug delivery, we used a two-compartment PK model [31] and simulated the plasma PK of NAL following release from the patch (Fig. 2d). The following system of equations describe the PK model integrated to the drug release model via the previously calculated spline interpolant of NAL flux (\( J_{\text{NAL}}(t) \)).

\[ V_c \cdot \frac{dC_{\text{NAL}}}{dt} = J_{\text{NAL}}(t) \cdot S_{pore} \cdot \left( \dfrac{1}{\Delta z(t)} \right) \cdot (1 \cdot \left( \dfrac{\text{Dose}}{N} \right) - M(t)) + Q \cdot C_{\text{NAL}} - (\text{Cl} + Q) \cdot C_{\text{NAL}} \quad \text{at } t = 0 \]  

(10)
Table 2 List of model parameters

| Parameter              | Definition | Value     | Units     | Ref |
|------------------------|------------|-----------|-----------|-----|
| Patch-related parameters |            | P1        | P2        | P3  |
| $r^*$                  | Base radius of MN | 0.01      | cm        | Exp |
| $h^*$                  | Height of MN | 0.047     | 0.068     | 0.0561 | cm  | Exp |
| $\theta$               | Half angle of cone apex | 12.01    | 8.37      | 10.1 | Degree | Calc |
| $N^*$                  | Number of MNs in a patch | 100      | 100       | 225  | -    | Exp |
| $\rho^*$               | Material density of patch | 1.5e+6   | $\mu$ g/cm$^3$ | [26] |
| $c_v^*$                | Solubility of patch matrix | 1.6e+5   | $\mu$ g/mL | Exp |
| $w^*$                  | Pitch of the patch | 0.0482   | 0.0488    | 0.0476 | cm  | Exp |
| **Dose**               | Dose of drug loaded in the patch | 17,895.94 | 17,203.9 | 17,814.45 | $\mu$ g | Exp |
| $\beta^*$              | Drug loading fraction of patch | 0.0715  | 0.0686    | 0.0708 | -   | Calc |
| $k_D^*$                | Dissolution constant of polymer | 0.001   | 0.0038    | 0.0064 | cm/h | Est |
| Th                     | Thickness of patch baseplate | 0.26    | cm        | Calc |
| S                      | Total surface area of patch | 0.64    | $cm^2$    | Exp |
| $S_{pore}$             | Porous surface area of skin | 0.0314  | 0.0314    | 0.0707 | $cm^2$ | Calc |
| $V_{patch}$            | Volume of patch | 170      |          |      |      |      |

Clinical pharmacokinetic parameters of NAL

| Cl                     | Total body clearance of NAL | 207 | L/h | [31] |
| Vc                    | Volume of distribution of NAL in central compartment | 12.1 | L | [31] |
| Vp                    | Volume of distribution of NAL in peripheral compartment | 102 | L | [31] |
| Q                     | Intercompartment mass transfer rate | 284.4 | L/h | [31] |

\[
\text{where, } l\in [\text{Dose}/(J_{NAL}(t)\cdot S_{pore}),(t)] = \begin{cases} 
1, & t \leq \text{Dose}/(J_{NAL}(t)\cdot S_{pore}) \\
0, & t > \text{Dose}/(J_{NAL}(t)\cdot S_{pore}) 
\end{cases}
\]

\[
V_p \cdot \frac{dC_{NAL,p}}{dt} = Q \cdot (C_{NAL,c} - C_{NAL,p}) \cdot C_{NAL,p}(0) = 0 \quad (11)
\]

Here, $C_{NAL,c}$ and $C_{NAL,p}$ represent the concentration of NAL in central (i.e., plasma) and peripheral compartments, respectively; $V_c$ and $V_p$ are the volumes of distribution of NAL in central and peripheral compartments, respectively; $S_{pore}$ represents the porous surface area of skin caused by the penetration of MNs, such that $S_{pore} = N \cdot \pi \cdot r^2$; Cl is the clearance of NAL from central compartment, and $Q$ is the flow rate characterizing the exchange of NAL between central and peripheral compartments. Model parameters are given in Table 2. The model was solved numerically as an initial value problem in MATLAB R2018a using the built-in function `ode45`.

Parameter analysis

To understand the effect of model parameters (physical and chemical properties of the MN patch) on plasma bioavailability of NAL, local sensitivity analysis (LSA) and global sensitivity analysis (GSA) were performed, using established methods [31–33]. For both GSA and LSA, the model parameters were perturbed within a ±50% range of the reference parameter values (given in Table 2), except for parameter $w$, i.e., pitch or distance between needles (varied between −37% and +50%). Note that the lower bound for $w$ was decided to ensure that pitch is never lesser than the diameter of the cone base.

In LSA, model parameters were perturbed one at a time at 1000 linearly spaced intervals between the previously defined range. For each perturbation, the PK model was solved to calculate the area under the curve (AUC$_{0-1}$) of the NAL plasma kinetics between 0 and 1 h. Note that since we are interested in rapid delivery of NAL, we ignore the long-term PK of NAL in our analysis. AUC$_{0-1}$ was estimated numerically via the trapezoidal method, using the built-in MATLAB function `trapz`. The qualitative nature of the relationship between NAL bioavailability and %perturbation was thus obtained.

In GSA, all model parameters were perturbed simultaneously to comprehensively evaluate and rank order the parameters for their effect on NAL bioavailability. For this, Latin hypercube sampling (LHS) [32–34] was used to obtain 1000 unique combinations of model parameters, and 10 such replicates were generated. Multivariate linear regression analysis was then performed on each replicate, and regression
coefficients were determined as a measure of sensitivity index (SI) for each parameter. A distribution of regression coefficients (or SI) was obtained for each parameter from the 10 replicates, and one-way ANOVA with Tukey’s test was used to rank the parameters in terms of their sensitivity, such that a higher SI represents a greater influence on NAL bioavailability.

**Statistical analysis**

Data was analyzed using Microsoft Excel and GraphPad Prism 8.4.3.686 software package. Student’s $t$ test, Shapiro Wilk test (normality evaluation), one-way ANOVA with unpaired $t$ test, and Welch’s correction (multiple test group comparisons) were done. Significant difference was concluded between test groups with “p” values < 0.05.

**Results and discussion**

**Solubility study**

The effectiveness of polymer strength of about 10% for PVP has been shown in a previous study. Though higher strength PVP was established to have faster dissolution rate, they were limited by skin insertion efficiency. MNs made with about 10% PVP were found to permeate the skin better [35]. The poor permeation property and weak penetration of higher strength PVP was attributed to its high-water adsorbing property, requiring the need for critical evaluation of the strength to be included in formulation while putting into consideration drug variables [35]. An initial evaluation by us using similar strengths of PVP for the fabrication of NAL MNs showed the efficiency of a 7.5% PVP formulation. The solubility of NAL in this PVP strength was found to be 159.08 ± 1.84 mg/ml. PVP was selected as the polymer for consideration because of its rapid dissolution property with the aim of meeting the current non-invasive and fast delivery need for NAL. It is a promising polymer, approved by the FDA for controlled drug release alongside other uses. More so, it has good biophysical characteristics ranging from good water solubility, tolerance to thermal treatment, great adhesion quality, biocompatibility, and safety. It is also cleared by the kidneys and no hazardous wastes are introduced into the environment [36–38]. Typical of dissolvable polymeric polymers, it also has a high drug loading capacity for hydrophilic drugs [38, 39] which is evident in the amount of NAL dissolvable in the polymer strength selected. Numerous studies have demonstrated its effectiveness as a polymer for MN fabrication for a couple of drugs, both lipophilic and hydrophilic ranging from small molecules to biotherapeutics [37, 38]. Its ability to deliver high drug flux was reported by Gao et al. in a transdermal formulation [40]. Based on the general attributes of this polymer and its successful record from numerous evaluations, our expectations following the previous feasibility studies done in our lab on the MN-based transdermal delivery of NAL using solid MNs were that for a clinical translational purpose, the development of dissolvable MNs for NAL using PVP is a veritable idea. With this approach, the limitations of solid needles are ruled out and an easy and inexpensive fabrication process which could be adaptable to large-scale use of NAL MNs and ensure rapid delivery was envisioned.

**Fabrication of naloxone MNs**

Considering the high drug loading capacity of the PVP polymer and the high solubility of NAL in it, attempts were made to form high strength NAL patches with the aim of achieving fast delivery of larger amount. As such, large drug amount was loaded in needle tips and entire patch. Based on the findings from the drug loading evaluations, 50 mg/ml formulations were eventually selected as optimal loading strength for further evaluation and optimization because for other drug concentrations evaluated (from 60 to 150 mg/ml), a major challenge encountered was crystal formation and the formation of turbid final products with 100 mg/ml to 150 mg/ml strengths. This observation is critical as crystallization in transdermal drug formulations could affect product reliability, in that the amount expected to be delivered could be overestimated with low dose being released instead. Drug kinetics in the skin becomes unpredictable as solubility could be affected and transit or drug retention or migration time is affected. Hence, poor and erratic product performance is anticipated with such high strength formulations which is undesirable from a drug formulation and clinical safety perspective [41]. As shown in Fig. 3a–d, the amount of crystals formed were observed to decrease with decreasing drug strength in dried PVP microfabrication. Their formation was thus suggested to be due to oversaturation of the 7.5% PVP solution with NAL as drying progresses with evaporation of water content. The saturation limit was thus predicted to be about 50 mg/ml NAL in 7.5% PVP in the dry state since a clear patch was achieved with this strength as seen in Fig. 3e. With this strength of PVP and NAL, clear needles with good integrity were obtained. Stepwise method optimization revealed that a consistent fabrication result is achievable with the steps described for the
mold casting process in the “Sect. 3.2 which was applied all through our studies.

Due to vortexing/stirring to ensure uniform drug distribution and dissolution, despite sonication of drug in PVP solution, significant bubble generation is typical, and it is important to ensure complete degassing by vacuuming during fabrication as bubbles could affect needle integrity, form, and drug loading [42]. It could also influence drug release properties [43]. However, care needs to be taken regarding preventing polymer solidification and drying with prolonged handling during this degassing phase. More so, the final vacuuming step at a pressure of \(-0.6\) psi for less than \(2\) h is also considered optimal. Excessive or prolonged pressure application was observed to cause needle base diagonal cracks which could be due to fluid being pulled down with excessive pressure. Overall, the key factors observed to affect the formation of PVP-based NAL MNs ranged from the quantity of drug dissolved in polymer, polymer strength, vacuum time, and drying period. Hence, attention needs to be paid to these critical details and all discussed formulation considerations when being adapted to produce reliable and uncompromised products.

**Characterization of optimized patches**

**Bright field imaging**

For the different needle dimensions, uniform needle formation was observed as shown in Fig. 4a–c. MNs were conical in shape with sharp pointed tips. Morphological dimensions such as the average needle height, interspacing tip distance, and base diameter for all the fabricated MNs are reported in Table 3. Homogeneity in the morphology across MN arrays of different dimensions was reflected by the values obtained. Moreover, the needle dimensions overall were comparable to that of master mold structures [44, 45] and are within appropriate range identified in literature as what requires minimal insertion force to penetrate the skin as well as that required for painless use [45, 46]. To confirm that the drug gets released at a fast rate based on the rapid dissolution property of the PVP polymer, the ability of the needle tips to dissolve within \(2\) min of insertion in the skin was also evaluated. Figure 4d–f show the remnants observed under the microscope following skin insertion for \(2\) min, which were proportionate to the height of the needles evaluated.
Dye Binding and skin insertion efficiency

Thumb force was used in applying needles to dermatomed porcine ear skin for 2 min. Images showing methylene blue dye retained in porated holes are shown in Fig. 5 for all the different needle dimensions fabricated. The insertion efficiency (IE) of these was determined to be 94 ± 4.8%, 90.6 ± 1.69%, and 96 ± 1.29% for P1, P2, and P3 MNs, respectively.

The values obtained are consistent with reports on similar dissolving microneedles made with PVP and polyvinyl alcohol which allowed a penetration efficiency of close to 100% [47, 48]. The slight variations seen with insertion with different skin pieces could be related to variation in skin elasticity [49]. Another contributing factor could be the inability to maintain similar thumb force across multiple application times. In the eventuality of the clinical success of polymeric NAL MNs, the design of applicators to ensure uniform application force across skin area will be of good use [50]. The retention of dye in the pores indicates efficient skin penetration and the formation of microchannels for the passage or diffusion of drugs. This also further indicates that the needles have sufficient mechanical strength to withstand the insertion force applied manually [51].

Confocal laser scanning microscopy

Confocal microscopy study further confirmed the capability of formed needles to porate skin and to allow dye to rapidly diffuse through created microchannels. Within 1 min of applying fluorescein as a fluorescent dye for imaging, a total dye diffusion length of 150 ± 4.08 µm was observed for the 800 µm NAL MN, 130 ± 2.5 µm and 80 ± 0.1 µm for the 600 µm and 500 µm MNs, respectively, as shown in Fig. 6a–c. There is evidence to show that they all produced microchannels sufficient for the transport of drug. As noted

Table 3 Dimensions of NAL-loaded PVP microneedles

| Measurements (n=6) | P1 Vendors specification | P2 Vendors specification | P3 Vendors specification |
|-------------------|--------------------------|--------------------------|--------------------------|
| Height (µm)       | 470 ± 1.77               | 500                      | 680 ± 6                  | 800                      | 561 ± 2.5               | 600                      |
| Tip to tip distance (Needle Pitch (µm)) | 482 ± 6                  | 500                      | 488 ± 3                  | 500                      | 476 ± 11               | 500                      |
| Base Diameter (µm) | 200 ± 0.2                | 200                      | 200 ± 0.8                 | 200                      | 200 ± 1.5              | 200                      |
by Marmato et al., MNs usually penetrate 10–30% of their actual length in the skin. This thus explains the different diffusion depth observed with confocal microscopy for the different needles which falls within this range. Typically, it is expected that an indent is initially created by needles in skin which dependent on pressure applied gets to break into the skin after a while during application [45, 52]. The depth of channel created then varies with the amount of pressure applied, skin viscoelasticity, and integrity [45]. Also, needle height is seen to correlate with diffusion path with 800-µm needles showing deeper dye diffusion depth compared to the rest within 1 min. The measured dye diffusion depth for each of the dimensions of MNs is consistent with observations from previous studies involving PVP dissolving MNs in skin. Liu et al. reported a diffusion length of 200–400 µm following application of 500 µm PVP MNs preloaded with fluorescein isothiocyanate for 15 and 30 min [53]. Likewise, following insertion in human foreskin Sun et al. observed a dye depth of 100 µm with fluorescently labelled PVP MNs of length 400 µm [38]. Also for 650 µm PVP MNs a study reported an insertion depth of 200 µm [21, 27]. Therefore, the conclusion reached is that the PVP-based polymeric MNs for NAL are of good performance considering the evidence of channel creation in skin.

**Drug content**

For all drug formulations, it is essential to analyze the quantity of drug in final formulation to have an estimate of the exact amount being dosed. Various factors ranging from spillage during the mold filling step and drug instability in formulation to crystallization could significantly impact final drug content. For all microfabricated needles, the theoretical NAL content per mold is 22.5 mg. The experimentally determined percentage drug content was 83.2 ± 1.06% (17.89 ± 0.23 mg), 80.02 ± 3.6% (17.2 ± 0.77 mg), and 82.8 ± 2.3% (17.8 ± 1.01 mg) for P1, P2, and P3, respectively. In this study, explanations for the almost 20% drug loss in the optimized formulations are attributed to the spill of drug in polymer solution from micro-molds during the

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Fig. 5  
(a) Methylene blue dye retention on skin post needle insertion and removal (image taken with an iPhone 8 camera).  
(b) Methylene blue dye retention on skin post needle insertion and removal (image taken with a brightfield microscope ×40 magnification) for (1) P1, (2) P2, and (3) P3.
mold casting process, particularly, the degassing and vacuuming phase. Fabrication under controlled and robust conditions is anticipated to minimize such loss. However, the reported drug contents are considered to still be within narrow limits from the theoretical amount of drug envisaged to be incorporated in MN arrays. Also, uniformity in drug content across different arrays is seen with the insignificant variation seen across multiple arrays (SE (n = 4)).

In vitro permeation study

Passive permeation of NAL hydrochloride

Few studies have evaluated the transdermal delivery of NAL. For what it is worth, this route is established by these studies as a preferred route for the delivery of NAL considering the numerous advantages users of this drug could benefit from. Passive permeation of NAL via the skin was shown by these research studies to be quite deficient and this limitation is established to be due to the hydrophilic property of NAL which limits its capability to diffuse through the lipid bilayer of the skin [13, 54]. Similar to these previous findings, we also observed no significant drug flux with passive delivery (1.53 ± 0.83 µg/cm²/h over 24 h) as shown in Fig. 7a. This data compares to that on passive delivery from our previous study for which a flux of 1.75 ± 0.25 µg/cm²/h was recorded [13]. In this current study, no significant drug flux was seen in the first 1 h of passive delivery and a lag time of 62.057 ± 4.05 min was also observed. Hence, despite the presented advantages, such as minimal invasiveness, sustained delivery, improved bioavailability, and the provision of easy options to firsthand care givers for the management of NAL emergencies, the utmost desire remains rapid release, to avoid loss of patients prior to the onset of some of the benefits associated with the route. This requirement is justified by the need for urgent recovery when patients present with respiratory depression and the need for very rapid stabilization with NAL [55]. No form of delivery indeed beats the bolus delivery possible with the IV route; however, the development of delivery systems with properties quite similar to IV delivery or at least close enough to that seen with IM, SC, or IN dosing is considerable options.

Effect of MNs: passive Vs P1

As a significant improvement over the passive permeation data from the current study (p < 0.05), as shown in Fig. 7a, the fabricated PVP-based MN, P1 produced an average drug flux of 18.71 ± 3.24 µg/cm²/h and an average cumulative amount of 440.14 ± 29.93 µg/cm² over 24 h. Previous studies show that the major obstacle with the transdermal delivery of NAL has been the lag time to drug delivery which ranges between 1.6 and 11.5 h [13, 54, 56]. For a transdermal system being considered for use in opioid emergencies, rapid delivery in the first 1 h of drug application is considered more critical to enable quick reversal of opioid complications. As shown in Fig. 7b, the treatment of dermatomed porcine ear skin resulted in higher permeation (p < 0.05) of NAL with P1 in the first 1 h at the respective time points over zero permeation amount seen with passive permeation at these time points. The average flux over the first 1 h with P1 was also 15.09 ± 7.68 µg/cm²/h. As was envisaged prior this study, the overall data with P1 reflects significant permeation enhancement and a reduction in lag time to 5 min (~90%) (p < 0.05)
over what was observed with the passive delivery of NAL. Also, compared to the 8-min delivery lag time previously reported in preliminary studies from our lab with the use of solid MNs [13], the 3 min difference seen in this current study with dissolvable MNs is considered significant and should make some difference with the onset of drug effect when used in vivo. Typically dissolving MNs create micro-depots of drug in deeper skin layers through the channels they form which first allows for rapid delivery into the receptor as drug diffuses through a shorter distance to the receptor. More so, release for a prolonged time is sustained from these drug depots in the skin as they are a repository for continuous release [57–62]. For a patch design with drug loaded in base, continuous flow from the base through already created channels into the receiving chamber is also expected. This explains the rapid and steady release of NAL seen over 24 h with the microfabrication. It is thus anticipated that the need for multiple injections to stabilize patients seen with existing parenteral forms of NAL can be eliminated using NAL dissolving MNs. This design is believed to be of greater benefit over the use of solid MNs previously evaluated by us since drug is loaded in the needle itself. The time taken to initially apply solid needles before applying drug is ruled out as drug dissolves in skin with insertion and there is no hazardous waste generated with this. More so, the risk of needle breakage in the skin is eliminated [21, 63–66]. Overall, the applicability of these MNs for the rapid delivery of NAL in clinical overdose presentations is thus evident.

Effect of MN length: P1 Vs P2

Interest in options to improve on drug flux particularly in the first 1 h led to the evaluation of the effect of increasing MN length on the permeation of NAL. The patch P2 (Table 1) consisted of an array of MNs with length of 800 µm (300 µm greater than P1), while all other fabrication parameters held constant. As shown in Fig. 7a, the overall permeation profile of P2 over 24 h was significantly higher ($p < 0.05$) than that of passive delivery and P1. Also, as shown in Fig. 7c, an exciting observation for P2 in the first 1 h was a significant increase ($p < 0.05$) in drug flux and amount permeated across all individual time points evaluated compared to what was obtainable with P1. The average drug flux and amount permeated in 1 h with P2 were $60.44 \pm 22.6568$ µg/cm²/h and $51.5 \pm 12.41$ µg/cm², respectively ($p < 0.05$), which are evidently higher than the values recorded with P1. Data from the confocal study with the creation of deeper diffusion depth by P2 explains the difference seen reflecting the creation of deeper channels by longer needles and hence higher permeation in same time points. Data from this study compares to that from similar studies where needle length has been shown to correlate with the depth of permeation and
amount permeated [67]. In the previous study by us, 250 µm MNs produced shallow channels in comparison to 500 µm MNs and this as well reflected a reduced permeation in comparison with the former, with longer length [13]. Banga et al. also made a similar observation with maltose MNs, for which depth of channels created was found to increase with increasing MN length and this as well reflected in the amount of loaded drug permeated [45].

**Effect of MN length and density: P1 Vs P3**

The effect of increasing needle density, i.e., number of MNs per unit area with a slight increase in MN length on drug flux and amount permeated, was also evaluated. As shown in Fig. 7d, P3 enabled much more significant amount of permeation compared to P1 over 24 h (p < 0.05). It was observed as well that within the first 1 h of application on dermated porcine skin, the average flux for P3 within 1 h was $102.83 \pm 32.34 \mu g/cm^2/h$ which is almost double with that seen with P2 and about sevenfold the flux seen with P1 (p < 0.05, Fig. 8). Table 4 summarizes in more details the 24-h flux and permeation profile of all three NAL MN microfabrications. The percentage permeated per square centimeter is 2.4, 10, and 23% after 24 h for P1, P2, and P3, respectively. The amount of drug remaining in patch is an indication of the prospect of using such MN patch for more prolonged release of NAL for about 4 days for the best performing patch (P3). The permeation increase seen with increased needle density is consistent with reports from previous studies where increase in permeation with increase in MN density is attributed to generation of more holes in skin [68, 69]. Though some variations were seen across samples as is evident in the SE of measurements, Lahiji et al. ascribes variations seen across sampling with skin pieces involving MNs to the difference in skin elasticity across skin pieces used in the studies as well as the amount of hair retained on skin post shaving which could influence penetration depth for each MN on an array across samples [49]. Also, as previously discussed, variation in pressure at the point of needle application could result in varied results across skin samples [45].

The results from this study evaluating the influence of MN length and density on NAL permeation/flux through the skin indicate that both factors significantly impact the amount of drug deliverable. Increasing MN density shows more promising influence with the same strength of drug (50 mg/ml). Therefore, it can be concluded that creating more channels on the skin for drug to diffuse through a larger area ensures a higher drug diffusion rate compared to increasing channel depth in the skin using longer length needles. This observation was as well seen with the data observed with confocal imaging studies; though longer length needles produced deeper diffusion length within 1 min of dye application, the difference seen with the slightly shorter length larger array needle was not so significant. Again, this could be attributable to skin elasticity, preventing the full length of the longer needle from getting into the skin. As seen with the 2-min post dye insertion images for P2, longer remnants’ post patch removal is shown in Fig. 4e. This indicates that there might be a limit to the yielding capacity of skin elasticity as MN goes in perpendicularly. Overall, both the in vitro permeation studies and needle characterization studies confirm the effectiveness of the polymeric needles for porating the skin to create transient channels for the diffusion of drug compared to an intact skin surface where the hydrophilic drug is limited from crossing the rate limiting barrier SC [19, 51]. The feasibility of manipulating patch array density and length to achieve significant therapeutic doses of the drug is also seen.

**Mathematical modeling and simulation**

**Model development and clinical pharmacokinetic predictions**

The drug release model (Eqs. (8) and (9)) was fit to in vitro drug release kinetics data for the three patch designs studied here (Fig. 9a), and the unknown model parameter $k_D$ was thus estimated for each design (Table 2). Since the region of interest for application in opioid overdose treatment is the first few minutes to an hour, the model was thus calibrated to reproduce the short-term (up to 1 h) experimental behavior only. As shown in Fig. 9a, the numerical solution
of the model is in good agreement with the data for all three patch designs, as also indicated by the Pearson correlation coefficient $R > 0.99$.

From the cumulative mass kinetics profile, NAL flux \( J_{\text{NAL}}(t) \) was calculated and spline function was fit to it, which showed good qualitative agreement with the experimentally calculated flux (Fig. 9b). The spline interpolant \( J_{\text{NAL}}(t) \) was then used to predict the plasma PK of NAL for all three designs using a two-compartment PK model (Eqs. (10) and (11)), and as shown in Fig. 9c, the P3 design which produced the highest flux led to the greatest bioavailability of NAL, followed by P2 and P1 patches. However, the plasma concentration achieved with the current designs is much lower than the pharmacologically effective NAL concentration value of ~0.34 ng/mL [13], and thus optimization of patch design is necessary to achieve the minimal effective concentration, or to match the performance of FDA-approved devices for NAL delivery.

**Model-guided patch optimization**

To understand the impact of model parameters on drug delivery and plasma bioavailability of NAL, we performed LSA and GSA with eight model parameters and assessed the effect of parameter perturbations on area under the plasma concentration kinetics curves during the first 1 h (AUC\(_{0-1\text{h}}\)). As shown in Fig. 9d, parameter perturbations between ±50% of the baseline values led to the greatest change in AUC\(_{0-1\text{h}}\) in case of the MN cone radius $r$ and MN count $N$, with both parameters showing a positive correlation with AUC\(_{0-1\text{h}}\). The remaining parameters showed only mildly positive correlations with AUC\(_{0-1\text{h}}\), except for patch material density $\rho$ that appears to have no impact on NAL bioavailability and pitch $p$ that was mildly negatively correlated to AUC\(_{0-1\text{h}}\). Of note, MN height $h$ only mildly affects AUC\(_{0-1\text{h}}\), which can be attributed to the model assumption that needles penetrate deep enough to be close to the microvasculature in the dermis of the skin. However, future analysis is necessary to incorporate the effects of mechanical strength of MNs and skin elasticity to estimate the true depth of MN penetration for a given height $h$. The polymer-related parameters, i.e., solubility $c_s$ and dissolution constant $k_d$, also positively affect AUC\(_{0-1\text{h}}\), thereby providing opportunities to enhance NAL bioavailability by modifying polymer composition. Also, drug loading fraction $\beta$ is mildly positively correlated with AUC\(_{0-1\text{h}}\), indicating that higher drug loading fraction can be considered to enhance NAL bioavailability, as long as it does not negatively impact MN strength. These observations suggest that increasing MN count and MN base diameter can strongly, positively impact NAL bioavailability, which can further be improved by keeping a shorter pitch, i.e., a higher MN density, as also corroborated by our experimental findings.

We further confirmed these observations through GSA, where model parameters were simultaneously perturbed to evaluate their impact on NAL bioavailability and also to understand the impact of potential parameter interactions. As shown in Fig. 9e, parameter ranking order from GSA validates the findings of LSA indicating that $r$ and $N$ are the most potent parameters in affecting NAL AUC\(_{0-1\text{h}}\).

As a result, we used these two parameters to optimize our best performing patch design (P3) to match the clinical PK of NAL obtained via FDA-approved intranasal (IN) and intramuscular (IM) devices [70]. As shown in Fig. 9f, the PK model was fit to the combined IN and IM clinical data to optimize parameters $r$ and $N$, while all other model parameters were fixed at the baseline values for the P3 patch. Our results indicate that the best fit of model predictions with clinical data was obtained for $r = 0.0238$ cm and $N = 2704$ needles (i.e., $52 \times 52$ array). Thus, increasing the P3 patch to an area $S$ of ~6.4 cm$^2$ with MN base dimensions to achieve $S_{\text{pore}} \approx 4.8$ cm$^2$ while keeping the other parameters unchanged can reproduce the clinical PK profile of NAL achieved through industry standard devices. Note that $\beta$ was held constant at 0.0708 (i.e., ~7% drug loading by weight) in our optimization; thus, the loaded dose in the optimized patch is ~185 mg.

Of note, as shown in the inset in Fig. 9f, not only does the optimized patch design matches the initial clinical performance of gold-standard devices, it also produces a long-lasting effect due to plasma concentration levels of NAL remaining above the effective concentration for up to ~7 h post administration. It can thus be inferred that increasing MN count while also increasing MN base diameter is an effective way to enhance the porous fraction of the skin.

### Table 4 Permeation profile of varying NAL MN fabrications

| Code for fabricated MNs | Array size (# of needles) | Theoretical dose in entire patch (µg) | Experimentally determined dose in patch (µg) | Average flux in 24 h (µg/cm$^2$/h) | Average cumulative permeation after 24 h (µg/cm$^2$) | Percentage permeated after 24 h per sq. cm (%) |
|-------------------------|----------------------------|--------------------------------------|---------------------------------------------|--------------------------------|---------------------------------------------|------------------------------------------|
| P1                      | 100                        | 21,500                               | 17,890±0.23                                 | 18.71±3.24                      | 440.14±29.93                                | 2.4                                     |
| P2                      | 100                        | 21,500                               | 17,200±0.77                                 | 71.90±22.67                     | 1731.87±299.82                              | 10                                      |
| P3                      | 225                        | 21,500                               | 17,800±1.01                                 | 178.83±76.26                    | 4112.89±773.40                             | 23                                      |
under impact by the patch, thereby leading to enhancement in drug flux and plasma bioavailability. Importantly, our modeling-based predictions are in concordance with evidence in the literature, where increase in MN density [71–73] and increase in MN base width [72, 74, 75] were found to correlate with an improvement in drug delivery.

**Conclusion**

The prospects of successfully applying PVP-based MNs for delivering naloxone in opioid emergencies have been demonstrated in this study. In vitro to in vivo mathematical extrapolation of the release profile of the best performing patch from this study has helped to identify significant parameters to be optimized in order to reproduce the clinical PK profile of the FDA-approved IN and IM NAL formulations. These include an increase in the diameter of the base of fabricated MNs and needle count. Ultimately, the applicability of rapidly dissolving NAL PVP-based MN for either the stabilization of opioid overdose patients or for maintenance therapy following stabilization to eliminate opioid-induced side effects such as pruritus and constipation is foreseen.

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

Declarations

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