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Individually coated microneedles for co-delivery of multiple compounds with different properties

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Abstract

Microneedle (MN) patches provide a simple method for delivery of drugs that might otherwise require hypodermic injection. Conventional MN patch fabrication methods typically can load only one or possibly multiple miscible agents with the same formulation on all MNs, which limits the combination and spatial distribution of drugs and formulations having different properties (such as solubility) in a single patch. In this study, we coated MNs individually instead of coating all MNs from the same formulation, making possible a patch where each individual MN is coated with different formulations and drugs. In this way, individually coated MN patches co-delivered multiple agents with different physicochemical characteristics (immiscible molecules, proteins, and nanoparticles) and in different spatial patterns in the skin. MN loading was adjusted by modifying the number of coating layers, and co-delivery of multiple agents was demonstrated in porcine skin. We conclude that individually coating MNs enables co-delivery of multiple different compounds and formulations with needle-by-needle spatial control in the skin.

Keywords

microneedle patch; co-delivery of compounds; transdermal drug delivery; immiscibility; coating; controlled release; programed release

Introduction

Transdermal drug delivery has attracted researchers' interest for several decades, since this manner of topical delivery is generally user-friendly and easy of terminate, which can improve patient acceptance and compliance [1–4]. Transdermal delivery also allows drugs to enter the systemic circulation while avoiding the first-pass effect in oral administration and the inconvenience of parenteral administration by injection. However, there is a significant limitation to traditional transdermal delivery: it can only effectively deliver small-sized, lipophilic molecules, due primarily to both the physical barrier of brick-and-mortar structure and the lipophilic microenvironment of the lipid matrix in the stratum corneum.

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Conflict of interest

Mark Prausnitz is an inventor of patents licensed to companies developing microneedle-based products, is a paid advisor to companies developing microneedle-based products, and is a founder/shareholder of companies developing microneedle-based products (Micron Biomedical). This potential conflict of interest has been disclosed and is managed by Georgia Tech and Emory University.

To overcome this obstacle, several techniques have been developed to improve penetration of drugs, such as penetration enhancers [5], thermal ablation [6], iontophoresis [7], and microneedle (MN) patches [8]. Among them, the MN patch technique has been proven effective for delivery of various therapeutic agents including drugs, vaccines, and even particles [9–16]. Meanwhile, the microscopic size of MNs results in minimal changes to skin structure that can repair within hours to days, which decreases the risk of exposure to the environment [17, 18].

Drug-loaded MN patches usually contain the drug coated on the MN surface or encapsulated within a dissolvable MN [8, 19, 20]. Coated MNs have been widely studied and use coatings made of water-soluble materials such as sucrose, carboxymethyl cellulose, and polyvinylpyrrolidone (PVP) [21–24]. Coated MN patches are typically made by repeatedly dipping MNs into a bath containing a homogenous solution or dispersion to build up a coating matrix on the MN surface; such patches can be utilized to load only one or multiple miscible agents. Therefore, this fabrication method presents difficulties in precisely controlling the composition of each individual MN on one patch, and imposes limitations on the choice of drugs. Moreover, it hinders the development of MN patches particularly for delivering multiple agents or formulations with different properties, such as immiscibility. One approach addresses this concern: incorporating immiscible agents in one heterogeneous vehicle, such as using liposomes [25–27] or layer-by-layer assemblies [28–30], but this requires more complex formulations and faces challenges in effective loading and stability of these vehicles.

Conversely, if each MN on a patch were made from different formulations, one individual patch could consist of MNs with multiple different loadings, and therefore has potential to load as well as co-deliver multiple different drugs, as well as to control the spatial distribution and delivery of the drugs in the skin. Typical coated MNs are made by coating the therapeutic components on pre-made metal MNs [22, 31–33] by either coating all the MNs in a patch at the same time, or by coating one MN at a time, often using an automated device. With individual coating, it is possible to make a coated MN patch where each individual MN is independently coated with a different formulation (Fig. 1). In this way, multiple drugs and formulations may be loaded and co-delivered to the skin. Moreover, by selecting which MNs receive each of the different coating formulations, a spatial pattern of the different formulations can be made and transferred to the skin to promote or inhibit interactions between the different formulations or otherwise to distribute the formulations to different locations on the skin.

In the present work, individually coated MN patches were developed to load and co-deliver multiple different formulations (immiscible dyes, dye-labeled protein, and nanoparticles). These compounds were chosen as model drugs or nanoparticles to simulate various molecules or formulations and were coated separately on individual MNs to assemble MN patches loaded with different combinations of these agents. The color or fluorescence patterns of the MN patch could be designed by choosing the coating sequence and dye combination. These MN patches were utilized to simultaneously deliver those dyes into skin and leave the corresponding patterns after a single application. After administration, the dye

species diffused, gradually changing the inserted pattern according to the formulation design; these patterns were recorded and studied using imaging software.

Experimental methods

Preparation of coated microneedles

Arrays of solid metal MNs were made by wet-etching photolithographically defined needle structures from stainless steel sheets (Tech Etch, Plymouth, MA). Each MN array consisted of 5 MNs, and each MN measured 750 μm in length and 200 μm $\times$ 50 μm in cross-section; this configuration has been previously described [22]. To coat different agents on the MNs, whole arrays were treated with oxygen plasma (Plasma cleaner PDC-32G, Harrick Plasma, Ithaca, NY) in advance to generate a more hydrophilic surface on the MNs, and then placed on a custom-made, automated coating station [22] using a two-axis robotic control so that the MNs could be dipped into a coating solution multiple times. Using an automated robotic coating process, individual MNs were separately coated, and therefore each MN could be coated independently with desired components. The arrays were allowed to dry overnight at room temperature (20 – 25 $^{\circ}\text{C}$) in a desiccator.

The coating solution contained 10% (w/w) polyvinylpyrrolidone [PVP K-15 (average molecular weight of 1×10^4 Da) or K-30 (average molecular weight of 4×10^4 Da), Spectrum Chemical, Gardena, CA], which has good solubility in water and works well as a coating matrix. The coating solution also contained different dye species which were coated individually on MNs in designed combinations and sequences to generate the desired pattern on MN patches: (i) water-soluble small molecule drugs were simulated by either fluorescein sodium salt (Fluo) or sulforhodamine B (SRhB); (ii) water-insoluble small molecule drugs were simulated by oil blue N (OB); (iii) macromolecular or protein drugs were simulated by fluorescein isothiocyanate-labeled bovine serum albumin (BSA-FITC), which was synthesized as described previously [34] with modification (see Supplementary Information [SI]); (iv) drug-loaded nanoparticles (NPs) were simulated by either color-dyed (red, blue, or yellow) polystyrene NPs (diameter 500 nm, Polybead, Polyscience, Warrington, PA), or fluorescent yellow-green polystyrene NPs (diameter 500 nm, Fluoresbrite, Polyscience, $\lambda_{\text{Ex}}/\lambda_{\text{Em}} = 441/486$ nm; see SI). Coating solutions were prepared in deionized water by dissolving PVP and dye species, except for the dye OB which was dissolved in ethanol.

Loading and release behavior of coated microneedles

By adjusting the number of dipping times, the loading amount on MNs could be varied and controlled. The relationship between the loading and the number of coatings was studied using NP-coated MN arrays. Using the method described above, MN arrays were coated with fluorescent NPs by being dipped into the coating solution between 1 and 20 times. The NP-coated MN arrays were then submerged in 1 ml phosphate-buffered saline (PBS), and kept on a shaking bed for 5 min to allow complete dissolution of the MN coatings. The PBS release medium was collected to measure the NP contents through fluorescence spectroscopy (Synergy H4 plate reader, Biotek, Winooski, VT).

The NP-coated MN arrays were also used to study the release kinetics of coatings from MNs. Several MN arrays were submerged in 1 ml PBS and kept for different durations. At each designed time point (5, 10, 15, 20, and 30 s, and 5 min), three MN arrays ($n = 3$) were removed, and their release medium was collected to measure the dye contents. The 5 min samples were considered fully dissolved and used to normalize other data.

Assembly of microneedle patches

The coated MNs were mounted on a small poly(methyl methacrylate) (PMMA) sheet to assemble MN patches. The PMMA sheet measured 1.5 cm in both length and width. Along the length of the PMMA sheet, 5 slots (measuring 1 cm in length) were engraved on the surface using laser cutting (Universal Laser, Scottsdale, AZ). These slots served as the mounting sites for MN arrays. These slots were filled with silicone (Sylgard 184, Dow Corning, Midland, MI) to hold the MN arrays in the slots. The assembled MN patches were stored in a desiccator at room temperature for one day to allow the curing of the silicone, which fixed the position of the MNs on the patches.

Application of microneedle patches on porcine skin

Porcine skin was obtained from a slaughterhouse, shaved with a razor and cleansed of subcutaneous fat. The skin surface was gently cleaned and dried with Kimwipes. The patch (MN side against skin) was pressed onto the skin with a single finger so that the MNs penetrated into the skin. The pressing was maintained for 5 s, and the patch was then left on the skin for 2 min to allow dissolution of the MN coating into the skin. The used microneedles were examined under a microscope (Olympus SZX16, Tokyo, Japan) to make sure they were not bent during application. The skin surface was cleaned with water and then imaged under the microscope (bright-field or fluorescence) for examination of the insertion sites and patterns. To observe the diffusion behavior of the delivered dye species, the bright field or fluorescence images of skin were taken at designed time points after applying the patches. For the experiments that took place over several hours, the skin was kept in a petri dish, and a small volume of PBS containing 0.02% sodium azide was added around the skin to hydrate the dermis and inhibit microbial growth. The skin surface was approximately 2 mm above the liquid surface.

As dyes diffused in the skin, the fluorescence intensity decreased at the original inserted sites, which were measured using ImageJ software [35]. A circle area (200 μm diameter) was selected at each inserted site on the images of skin, and fluorescence intensity was recorded over time by the software as the intensity of that chosen site. For each site, all intensities at different time points were normalized to an early time point (i.e., 3 or 5 min after insertion) to quantify its kinetic change.

Histological cross-sections of skin were observed where patches were applied that had been coated with both water-soluble SRhB and insoluble OB. At 30 min or 24 h after insertion, the skin was fixed and sliced along the direction of the MN array, and the bright field images were taken to compare the diffusion of SRhB and OB.

Statistics

The two-tailed unpaired Student's *t*-test was used to compare the data. All results were presented as mean $\pm$ S.D., and a difference of $p < 0.05$ was considered statistically significant.

Results and discussion

Individually coated microneedles

MN arrays are typically coated, either individually or all together, with a single coating solution, which can make it difficult to co-deliver compounds with different physicochemical properties. In contrast, if the MNs are coated individually, different compounds can be formulated individually and all distributed onto a single array of coated MNs (Fig. 2A), in contrast to coating all MNs with the same formulation (Fig. 2B). With the individual coating, coated MNs could be utilized for the co-delivery of multiple agents without requiring complex and sometimes biphasic formulation design and optimization.

In addition to controlling the type of compound and formulation on each MN, the amount coated on each MN can be controlled as well. During fabrication, dipping more times resulted in more materials being transferred from the coating solution to MNs, thereby increasing the drug loading on MNs. Shown in Fig. 3A, the loading gradually increased as more dipping times were used for coating. The relationship tended to be linear ($R^2 > 0.99$) when the MNs were dipped no more than 20 times. Moreover, the loading amount was also influenced by the matrix material; using PVP K-30 for coating led to a higher loading than using K-15 at the same coating condition. This was attributed to the higher molecular weight of K-30 than K-15, and therefore a more viscous K-30 solution, which facilitated the attachment of more materials on MNs during coating solution drying. In short, the results suggested that precise control of the loading on MNs could be possible by either adjusting the number of coating dips or the matrix materials.

Fast release of coated materials from microneedles

Different materials, such as saccharides, sodium carboxymethyl cellulose and other water-soluble polymers [36–38, 32], have been used as the coating matrix for coated MNs. Here, PVP was chosen as the coating material since it could be easily dissolved in an aqueous medium as well as many organic solvents. As shown in Fig. 3B, the PVP coating could be dissolved from the MNs within half a minute in PBS. Compared to coatings with PVP K-30, those with K-15 of lower molecular weight could be dissolved even faster—within several seconds. The fast release of the coated material from MNs is preferable for the practical insertion into skin, since it can reduce the required application duration of MN patches on skin to release the payloads, and thus increase the compliance of patients.

Assembled microneedle patches and application on porcine skin

Each MN array used in this study was etched from a thin metal sheet consisting of just five MNs, which were difficult to handle precisely. Thus, it was preferable to assemble several MN arrays into one patch that could be more easily handled. Here, as a patch prototype, a PMMA sheet was utilized as a MN array holder, which had engraved slots (1 cm in length

and 400 μm in width) to mount several MN arrays (Fig. 4B, C). Each MN patch included 5 arrays for a total of 25 MNs aligned in a 5×5 pattern. If needed, the distance between MNs, as well as the number of MNs on one patch, could be changed by adjusting the dimensions.

The MN patch simultaneously inserted all 25 MNs into skin and released the different payloads from each MN. As an example, in Fig. 4, red and blue dyed NPs were alternately coated on MNs, and were simultaneously delivered into skin after insertion. The alternating red/blue pattern generated in the skin corresponded to the coating pattern of the patch, indicating the co-delivery of both dyes. This result suggested that one patch could simultaneously deliver up to 25 different components, which may facilitate the co-delivery of multiple therapeutic agents or help control the release behavior of coated drugs.

Co-delivery of multiple compounds from coated microneedles

Several different dyes were used to simulate either free drugs or drug-loaded NPs. On each patch of 5×5 MNs, two or three dyes were coated on individual MNs, and were co-delivered into porcine skin upon application of these MN patches. After removing the patches, the dyes were released and remained in the insertion sites, and gradually diffused in the skin, which was observed under the microscope.

Co-delivery of water-soluble and insoluble agents—One MN patch design simultaneously delivered both water-soluble and insoluble dyes. As shown in Fig. 5A, the patch coated with water-soluble SRhB and water-insoluble OB delivered both dyes into skin, leaving a corresponding red/blue pattern in the skin. This was achieved by SRhB coating using an aqueous coating solution and OB coating using an ethanol-based coating solution. Within several hours, the red color gradually expanded, dimmed and disappeared from its insertion sites, attributable to the fast diffusion of water-soluble SRhB. Conversely, the blue color dots remained near their insertion sites even after four hours, since the water-insoluble OB formed dissolution-controlled release depots in skin. The co-delivery of two dyes, as well as their diffusion, was confirmed by histological examination (Fig. 5B); the red color of SRhB was no longer visible at the insertion site after 24 h, while the blue color of OB remained, revealing the difference in diffusion. This result suggests that, using coated MN patches, co-delivery of both water-soluble and insoluble drugs without mixing is possible, since these drugs could be coated and delivered independently.

Co-delivery of free molecules and nanoparticles—In addition to delivering free dyes, the MN patch was also examined to determine if it could simultaneously deliver NPs and free dyes. In one case, the patch was designed to deliver both green fluorescent NPs and Fluo, and showed an emerging “G” letter pattern in skin (Fig. 6A, B). Since the free dye diffused away much faster than the NPs, Fluo rapidly diffused away from its original sites after insertion, as shown as the rapid decrease of green fluorescence in the corresponding insertion sites. Within the same time period, NPs diffused much slower and their fluorescence intensity decreased less. After four hours, Fluo sites maintained $<10\%$ of original fluorescence intensity and could hardly be seen under the microscope. The NP sites still kept $>70\%$ of their fluorescence intensity, resulting in a “G” pattern. This result simulated the co-delivery of a free drug and its dosage forms (such as NPs), where two

components with different release rates were delivered at the same time. The fast-release component may generate a burst release to meet acute requirements, while the slow-release component may continue a sustained release. This raises the possibility that controlling the overall release profile may be accomplished with careful design of the composition and ratio between free drugs and drug-loaded formulations for coating.

In a similar experiment, the MN patch simulated co-delivery of a formulation consisting of a free drug and NPs loaded with another drug by coating with green fluorescent NPs and red fluorescent SRhB. As shown in Fig. 6C, D, the SRhB-coated MNs left a red “ π ” letter pattern in skin after applying the patch, and their insertion sites lost around 80% of fluorescence intensity after four hours, shown as the “disappearing π ” pattern. Conversely, the green NPs sites retained >70% of their original intensity. This result suggests that, in the case of co-delivery of two different drugs, controlling the release rate of either one is possible if appropriate dosage forms are selected.

Co-delivery of small molecules, macromolecules, and nanoparticles—A more complicated experiment was then designed using a single patch to co-deliver three agents of different sizes including free dyes, protein, and NPs. This combination contained both large and small molecules as well as particles and free drugs, which might be sufficient for almost any drug delivery system. In this experiment, the MNs were individually coated with green fluorescent NPs, BSA-FITC, or free Fluo, and were assembled into MN patches with designed patterns shown in Error! Reference source not found.. All three types of components were successfully delivered into skin after the MN patch was applied, and each component diffused at a different rate, changing the corresponding pattern in skin over time. As a free water-soluble dye, Fluo quickly diffused within several minutes, and its intensity dropped to <20% after four hours; the bigger molecule, BSA, moved more slowly in tissue, and its intensity slowly declined to ~40% after four hours; NPs had the slowest diffusion and retained an intensity of >70% over four hours (Fig. 6F). After a 2-day interval, only traces of Fluo and BSA remained, while NPs still maintained 40% of their fluorescence intensity, indicating good retention of the NPs at the delivery site.

Discussion

This study shows that coated MN patches effectively and simultaneously delivered drugs or particles of different sizes or solubility with a single administration. This individually coated MN design is straightforward since all components are loaded on MNs independently. Carrying out individual coating manually would be time consuming, but programming an automated robotic coating station would make individual coating relatively straightforward and time efficient.

Co-delivery of drugs from individual coated MNs could be particularly useful for simultaneous administration of vaccine with adjuvant. Most vaccines are only stable in an aqueous coating solution, but some adjuvants (e.g., imiquimod [39]) are poorly water-soluble and would be more easily coated using a coating solution made using an organic solvent. Another vaccination example would be the co-administration of free vaccine to serve as an immediate-release priming dose and vaccine encapsulated in a delayed-release

formulation to serve as the boosting dose [40]. Similarly, many drugs (e.g., acetaminophen [41]) would benefit from delivery of free drug to provide immediate benefit to the patient, followed by slow release of drug from a controlled-release particle formulation to provide ongoing therapy. Finally, many drugs are given as multi-drug cocktails that can have different stability profiles. Individually coated MNs allow each drug to be formulated using different excipients that maximize the stability of each drug.

For the coated MNs reported here, the dissolution of the MN coatings was fast enough to allow the simultaneous release of agents on different MNs. One benefit of the individually coated MNs is that co-delivery of multiple immiscible agents can be facilitated without incorporation into specific forms of multiphase mixtures or vehicles. Moreover, since the loading composition on each individual MN can be manipulated separately, it is possible to control the overall loading and delivery of the whole patch through specific adjustment of individual MNs. With careful adjustment of the coated formulations—such as free drug and drug-loaded NPs, including their concentration, coating times, numbers and ratios of different MNs—we hypothesize that a precisely controlled or programmed release platform could be achieved using individually coated MN patches. In addition to the optimization of formulations coated on MN patches, one also needs to consider the *in vivo* factors, such as the microenvironment in the skin and interaction with systemic blood and lymphatic circulation, when pursuing a controlled drug release system using MNs. Drug distribution in the skin would not only depend on blood flow and other factor found *in vivo*, but would also depend on drug physicochemical properties (e.g., diffusivity, binding to skin microstructures, uptake/partitioning into cells), which has been studied before [42, 43]. After the coated formulations were delivered into the skin by MN patches, the overall release behavior depends on the individual composition of different MNs, as well as the formulation of controlled-release particles or other components that are deposited in the skin, and the impact of surrounding tissues.

Individualized composition of different MNs on a single patch could also be applicable to dissolvable MNs. For dissolvable MN patches made by casting on specific molds, some high-throughput techniques such as ultra-low volume dispersion [44] may provide a method to precisely load each MN with different desired components.

Conclusion

In this report, the coated MN technique was studied for its potential to co-deliver multiple compounds with different properties. NPs, protein, and free water-soluble or insoluble dyes were chosen to simulate varying drugs or drug formulations. By loading these agents independently on individual MNs, the MN patches could simultaneously deliver them with a single administration to skin. This straightforward design makes it possible to assemble differentially coated MNs on a single patch. This not only provides a method for co-delivery of multiple immiscible agents, it also forecasts a potential drug delivery platform with precisely controlled release ability.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix: Supplementary information

Supplementary information to this article includes the preparation and characterization of BSA-FITC as well as the characterization of fluorescent NPs, which can be found online at xxxx.

Fig. 1. Schematic illustration of individually coated microneedles (MNs) for co-delivery of multiple different compounds and formulations. (A) MNs are individually coated with different drugs and/or formulations. (B) MNs are applied on skin to simultaneously co-administer the multiple drugs and formulations coated on MNs. (C) MNs are removed after co-delivery of all MN coatings.

Fig. 2.

Representative coated microneedle (MN) arrays. Photographic images of representative arrays of MNs (A) individually coated with five different-colored dyes and (B) all coated with the same dye. Coated dyes are 1) soluble crystal violet dye, 2) blue-dyed nanoparticles (NPs), 3) azure color from the mixture of blue-dyed and yellow-dyed NPs, 4) yellow-dyed NPs, and 5) red-dyed NPs.

Fig. 3.

Control of microneedle (MN) coating amounts and rapid kinetics of MN coating release in skin in vitro. (A) The linear relationship between loading amount on MNs and number of times MNs were coated. Data show mean $\pm$ S.D. ($n = 4$). The inserted images showed the individual MN with 5, 10, 15, and 20 coats; the scale bar is 200 μ m. (B) The release profile of nanoparticles from coated MNs (number of coats = 15) in phosphate-buffered solution. Data show mean $\pm$ S.D. ($n = 3$). The coating matrix is PVP K-15 or K-30.

Fig. 4.

Representative photographic images of assembled microneedle (MN) patches and application to porcine skin. (A) Image of two linear MN arrays individually coated with alternating red or blue nanoparticles in different sequences. (B) Image of PMMA sheet with five empty slots (indicated by arrows) to form a patch backing (top view). (C) Image of a patch containing a 5×5 array of MNs assembled using five linear MN arrays (e.g., panel A) in a PMMA patch backing (e.g., panel B). (D) Image of porcine skin ex vivo after insertion of a MN patch (e.g., panel C), showing the alternate red and blue pattern in the skin (top view). The scale bars are 2 mm (A) and 5 mm (B, C, and D).

Fig. 5. Co-delivery of water-soluble and insoluble compounds in skin by a single microneedle (MN) patch. (A₁) Schematic pattern design of MN patch coated with water-soluble sulforhodamine B (SRhB) and water-insoluble oil blue (OB). (A₂–A₆) Representative photographic images of porcine skin ex vivo (top view) showing the kinetic color change after applying a MN patch with the pattern shown in panel A₁. (B₁–B₂) Representative histological images of cross-sections of skin 30 min (B₁) and 24 h (B₂) after MN patch insertion showing that SRhB (red arrow) diffused away while OB (blue arrow) remained near the insertion point for more than one day. The scale bars are 5 mm (A) and 200 μ m (B).

Fig. 6. Co-delivery of free molecules and nanoparticles (NPs) in skin by a single microneedle (MN) patch. Schematic images showing the 5×5 pattern of MNs in a patch coated with (A₁) free fluorescein sodium dye (Fluo) and fluorescently labeled nanoparticles (NPs), (C₁) free sulforhodamine B dye (SRhB) and fluorescently labeled NPs, and (E₁) free Fluo dye, free bovine serum albumin labeled with FITC (BSA sites) and fluorescently labeled NPs. Representative photographic images of porcine skin ex vivo (top view) showing the kinetic color change after applying a MN patch showing (A₂–A₆) the “emerging letter G” pattern, (C₂–C₆) the “disappearing letter π” pattern and (E₂–E₆) the disappearance of each of three different compounds over different time scales. The scale bar is 5 mm. Kinetic color change in panels (A, C, E) is quantified in panels (B, D, F), respectively. Data show mean ± S.D. ($n = 4$).