Smart Mushroom-Inspired Imprintable and Lightly Detachable (MILD) Microneedle Patterns for Effective COVID-19 Vaccination and Decentralized Information Storage

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ABSTRACT: The key to controlling the spread of the coronavirus disease 2019 (COVID-19) and reducing mortality is highly dependent on the safe and effective use of vaccines for the general population. Current COVID-19 vaccination practices (intramuscular injection of solution-based vaccines) are limited by heavy reliance on medical professionals, poor compliance, and laborious vaccination recording procedures, resulting in a waste of health resources and low vaccination coverage, etc. In this study, we developed a smart mushroom-inspired imprintable and lightly detachable (MILD) microneedle platform for the effective and convenient delivery of multidose COVID-19 vaccines and decentralized vaccine information storage. The mushroom-like structure allows the MILD system to be easily pressed into the skin and detached from the patch base, acting as a “tattoo” to record the vaccine counts in situ without any storage equipment, offering quick accessibility and effortless readout, saving a great deal of valuable time and energy for both patients and health professionals. After loading inactivated SARS-CoV-2 virus-based vaccines, MILD system induced a high level of antibodies against the SARS-CoV-2 receptor-binding domain (RBD) in vivo without eliciting systemic toxicity and local damage. Collectively, this smart delivery platform serves as a promising carrier to improve COVID-19 vaccination efficacy through its dual capabilities of vaccine delivery and in situ data storage, thus exhibiting great potential for helping to contain the COVID-19 pandemic or a resurgence.

KEYWORDS: bioinspiration, vaccine delivery, decentralized data storage, detachable microneedle, in situ labeling

INTRODUCTION

Thanks to enforced nonpharmacologically preventive measures (mask-wearing, physical distancing, hand disinfection, etc.) and effective clinical treatments, the spread of coronavirus disease 2019 (COVID-19) has slowed in certain countries and regions. However, it still remains one of the most concerning threats to human health globally, with about 500 million confirmed cases and over 6 million deaths up to now (April 2022), and the numbers are still growing at an alarming rate in some countries and regions. Currently, the widely accepted consensus is that the key to restoring the pre-epidemic normalcy relies largely on the timely and effective vaccination for the general population against COVID-19 according to the World Health Organization’s guideline.3−5

Since COVID-19 was confirmed to be caused by a recently discovered coronavirus (SARS-CoV-2 virus), numerous laboratories and companies have been racing to develop vaccines against COVID-19 and speeding up clinical trials all over the world.6 Encouragingly, approximately 300 vaccine products with promising potential are now under preclinical or clinical investigation.7 Several approved vaccines (inactivated SARS-CoV-2 vaccine (Vero cell) from Sinopharm, China
National Biotec Group Company; BNT162b2 mRNA vaccine from Pfizer-BioNTech; mRNA-1273 vaccine from Moderna, Inc., etc.) have displayed exciting efficacy with satisfactory safety, bringing great hope in containing the COVID-19 pandemic.6,7

Nevertheless, achieving general vaccination is still challenging, as current vaccination campaigns are limited by relatively poor recipients’ compliance and laborious vaccination procedures. Most clinically approved vaccines require two doses or even multiple doses, leading to a large number of people flocking to designated vaccination sites repeatedly to be vaccinated, which may diminish people’s enthusiasm, patience, and interests, therefore decreasing their compliance and increasing the risk of disease transmission as well as health workers’ burden. Moreover, it is usually time- and effort-consuming to accurately record and keep track of such a large amount of vaccination data, which inevitably causes mistakes due to highly frequent data access and storage. Another reason limiting broad vaccination is thought to be the strict cold-chain transport and refrigeration required for preserving solution-based vaccines, which impedes distribution and application of the vaccines, particularly in developing and tropical countries and regions.8,9 Thus, a strategy for effective, facile delivery and distribution of vaccines is highly desired toward containing COVID-19.

Compared to the conventional solution-based vaccines that are administered through muscle or deep subcutaneous injection by healthcare professionals, delivery and distribution of vaccines via microneedle patches may serve as a more appropriate vaccination strategy during the current COVID-19 pandemic. In our previous works, we have provided a broad spectrum of researches and overviews on microneedle-based systems for long-term subcutaneous/transdermal drug delivery and effective sample collection.10−12 Microneedle patches, consisting of an array of micron-sized needles and a base layer, can easily pierce the stratum corneum with a thumb press or an applicator to deliver vaccines into the skin. Such minimally invasive and painless vaccination procedure would improve recipients’ compliance.13−17 Additionally, microneedles store vaccines in an anhydrous form which has proven to reduce the reliance on the cold chain by restricting molecular vibration against heat.18 Moreover, they could release the vaccine by self-dissolving in response to interstitial fluid under the skin after administration, suggesting a facile and effective method.19−21 However, most currently used microneedle patches would attach to the skin with a base layer, which may be wetted by sweat or environmental liquid to increase infection risk, and also would result in patch loss during the scratch. Besides, regular patch-based vaccination would not ameliorate vaccine data storage to improve efficiency, encouraging scientists and engineers to develop a better vaccination strategy with both high vaccine delivering efficacy and easy data access capabilities.

Inspired by the natural structure of mushrooms, which consist of an umbrella-shaped cap with a thin stalk that not only can be firmly attached to the ground but also can be easily pulled off,22,23 here we designed a narrow neck that is located between the microneedle and patch base layer, which allows the patch base to be easily detached from the microneedles by water dissolving and pulling force, thus causing no discomfort to the vaccinee’s daily life and reducing risk of infection (Figure 1). The microneedles left in the skin would spontaneously release the vaccine via gradual degradation to

**RESULTS**

**Fabrication and Characterization of the MILD Microneedle Patches.** To fabricate the MILD microneedle patches, a traditional mold-based approach was adopted as shown in Figure 2A. A master mold with mushroom-like arrays was designed in Solidworks software and constructed by a 3D printer (Figure 2B). A negative poly(dimethylsiloxane) (PDMS) mold was built from the master mold by mixing

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two-part resin systems containing vinyl groups (part A) and hydrosiloxane groups (part B) at the ratio of 10:1 (Figure 2C), which perfectly maintained the mushroom-like shapes (Figure 2D). Alginate (ALG) and carboxymethyl cellulose (CMC) mixture at different ratios containing the vaccine was loaded into the PDMS mold with a loading efficiency of 93.5% (vaccine). No visible alterations in appearance and structures were observed in the MILD systems loaded with or without vaccines (Figure S1), and protein determination assay confirmed that the vaccine stored in the microneedles at room temperature was stable for at least 28 days (Figure S2). The patch base was formulated using 3% CMC, and the resulting MILD microneedle patch was obtained by carefully demolding from the PDMS (Figure 2A). To facilitate the separation of microneedle tips from the base layer, a narrow neck was created between the tip and base, resembling mushroom-like structures (Figure 2E,F). Scanning electron microscopy image demonstrated a textured and rough surface.
mainly attributed to polymer deposition during the drying process (Figure 2G). Moreover, the MILD system was able to readily penetrate an agarose gel (1.4%), and the base could be manually peeled off from the microneedle tips (Figure 1H–J).

Furthermore, a series of MILD microneedle patches with different sizes were fabricated, with the needle diameter ranging from 200 to 600 μm and the height ranging from 600 to 1000 μm (Figure S3). The mechanical strength of these MILD microneedle patches was measured using a tensile/compression machine, and the maximum tolerated compressive force was observed to be positively related to the needle height but not with the diameter, as the compression failure force of the medium microneedles (1.04 N per needle) was greater than the thick (0.88 N per needle) and thin microneedles (0.6 N per needle) (Figure 2K–M). This was possibly attributed to the balance between aspect ratio (AR) and neck burden (thin structure provided high AR, and thick one offered overload on the neck). To ensure highly effective skin penetration, the medium MILD patch was selected as the most favorable candidate for transdermal administration, which exhibited greater failure force than that was required for stratum corneum penetration (≥0.058 N) (Figure 2L).24 Besides, the compression failure force of the CMC-based microneedle was 1.41 N per needle, while the value of the alginate-microneedle patch was 0.684 N per needle (Figure S4), indicating that CMC dominates the mechanical strength over ALG to facilitate skin penetration without microneedle breaking. A mixture of alginate and CMC would not decrease the strength much under vertical press (Figure S4), but could slow down the dissolving speed of the tips (Figure S5) and consequently led to a sustained vaccine release over a week (Figure S6), which reportedly allowed more chance for the gradually released vaccine to interact with local dendritic cells and antigen-presenting cells.25

The tensile strength was evaluated after the microneedles were inserted into an agarose gel, followed by the slow elevation. Unlike compression strength, the base layer was readily pulled off from the gel surface (Figure S7), without
strong forces (Figure 2N–P). It should be noted that with the sizes increased (length and diameter), the required force to separate base layers were elevated accordingly, but all values were in low ranges (less than 0.12 N per needle), suggesting an effortless separation property of this mushroom-structural design.

**MILD Microneedle Pattern Design and Ex Vivo Labeling.** To record the counts of vaccinations, the MILD microneedle patches in a 3 × 9 array (in one building block) were arranged into Arabic numerical patterns (Figure 3A and B), which could in situ record the vaccine information. For example, the number “1” was made up of two blocks, while the
number “2” consisted of five blocks (Figure 3B). To fluorescently visualize the MILD microneedle patterns, crystal violet, a dye that emits a strong signal under 580 nm excitation without affecting vaccine stability (Figure S8), was applied as the indicator, endowing the MILD platform with in situ imprintable capabilities. Moreover, a small amount of polycaprolactone (PCL, molecular weight, 45 kDa, 2 mg/patch) was integrated into the MILD system to maintain fluorescence stability as a result of its relatively slow degradation.26 Expectedly, the MILD patterns presented an obvious fluorescent dotted matrix of Arabic numerical patterns under the excitation (Figure 3C). Subsequently, the crystal violet-loaded MILD microneedle patch was inserted into the agarose gel (mimicking tissues) to observe its tip-separable behaviors. After penetrating the surface of the gel and being wetted by water, the base layer was readily peeled off, leading to the quick “neck” break (Figure 3D), retaining all the tips within the gel (Figure 3E–G), suggesting effective base-tip separation. Additionally, the MILD systems were tested in the tissue sample (pig skin) to evaluate their applicable potential. As shown in Figure 3H,I, the microneedle was able to effortlessly pierce into the stratum corneum using thumb pressing (Figure S9). Notably, regular microneedle patches (conical shape) were completely retrieved from the skin.

Figure 5. In vivo immune responses to COVID-19 vaccine delivered by MILD microneedles. (A) Schematic illustration of mice receiving vaccination (free vaccine or vaccine-loaded MILD microneedles (MILD-vaccine)), the lymph nodes (LNs), spleen, and serum were collected at the given time points for immunological analysis. (B) The representative flow cytometric images of activated T cells (CD8+IFN-γ/IL-2+) in LNs isolated from the mice 24 days after being treated with PBS, free vaccine, or MILD-vaccine. (C) The representative flow cytometric images of memory B cells (CD19+CD27+) in LNs (upper panel) and spleen (lower panel) isolated from the mice 24 days after the given treatments. (D) IFN-γ levels in serum isolated from the mice 24 days after the given treatments. *p < 0.05, relative to PBS group. ANOVA. (E) Schematic illustration of the mice receiving two-dose of vaccines (free vaccine or MILD-vaccine), and blood was collected once a week for SARS-CoV-2 RBD antibody detection. (F) Schematic diagram of the two-dose vaccine triggering an immune response and producing specific antibodies against the virus. (G–I) The SARS-CoV-2 RBD antibody levels in each mouse (n = 4 mice per group). SARS-CoV-2 RBD antibodies were semiquantitatively detected with an ELISA kit and expressed by the ratio of sample serum optical density (OD) to that of the negative control (PBS group). Relative OD value ≥1.5 and <2, suspected positive; relative OD value ≥2, positive. (J) Titers of the SARS-CoV-2 RBD antibody in each mouse at week 5 and 6 after initial vaccination.
without any dye residue after removal (Figure 3H), demonstrating no in situ labeling capability. Impressively, the mushroom-like tips were retained in the microchannels due to the interlocking effect and fragile neck of the needles (Figure 3I). Moreover, the purple dotted pattern was clearly visualized with naked eyes due to the original color of crystal violet, serving as an ideal way for vaccination information imprint. Besides, when the skin treated by the MILD system was exposed to the exciting light, the marks were perfectly presented in “1” or “2” patterns with satisfactory signal-to-noise ratios (Figure 3J,K), suggesting a promising imaging profile.

In Vivo Vaccination Counts by MILD Microneedle Patterns. To assess the in vivo imprinting potential of the MILD microneedle patterns, the Sprague–Dawley (SD) rats were anesthetized, followed by hair removal and patch attachment (Figure 4A). Fluorescein isothiocyanate (FITC) was selected as the indicator as a result of its superior fluorescence quantum yield and high biocompatibility. A small amount of PCL (2 mg/patch) was encapsulated to extend the imaging time over 28 days under a living imaging system. The FITC-loaded MILD microneedle patterns were verified to be visibly excited at the wavelength of 490 nm with 100% of microneedle tips in high fluorescence intensity (Figure 4B,C). The patterns “1”, “2”, and “3” were inserted into the dorsal areas of different rats that were termed as “P1”, “P2”, and “P3”, respectively (Figure 4D). Importantly, the dotted fluorescent imprints were apparently observed on the dorsal area of the rats, and the numerical pattern remained intact for at least 28 days that is longer than the recommended interval (21 days) between two doses of the COVID-19 vaccine (Vero cell), and the patterns were effortlessly identified with intense signals (Figure 4E–J and Figure S10). Notably, the fluorescence intensity on day 28 was comparable to that of the pattern at the initial inoculation (Figure 4H–J and Figure S11), demonstrating the stable in vivo labeling capability of the MILD system, suggesting the possibility of long-term application. Moreover, based on this phenomenon, it is expected that the MILD microneedle pattern could offer more labeling in addition to dose counts, which help physicians and recipients gain more information during the vaccination process. In this regard, we successfully organized the blocks to present diverse patterns, ranging from numbers/letters to specific shapes, which could indicate more cues about the vaccination such as date, types, producers, and so on, after a rational combination (Figure 4K and Figure S12).

MILD Microneedle System Successfully Delivered COVID-19 Vaccine In Vivo. To determine whether the MILD microneedle patches were capable of delivering vaccines, inactivated SARS-CoV-2 vaccine (Vero cell) from Wuhan Institute of Biological Products Co., Ltd. and Wuhan Institute of Virology was encapsulated into the tips (MILD-vaccine), followed by the insertion in the right infra-axillary skin of the mouse model (Figure 5A and Figure S13). The original liquid solution-based vaccine formulation was set as a positive control, while PBS injection was used as the negative control. Lymph nodes, spleen, and serum were collected from the vaccinated mice for immunological analysis (Figure 5A). Compared to the PBS-treated mice, MILD-vaccine significantly increased the presence of IFN-γ- and IL-2-expressing CD8+ T cells in lymph nodes (LNs) by 2.4- and 3.4-fold, marginally higher than those of the free vaccine (1.8- and 2.6-
fold) (Figure 5B and Figure S14), which played a significant role in cellular immunity by actively eliminating the virus. Moreover, memory B cells, mainly accounting for the lasting humoral immune response against the virus, were enriched in LNs and spleen of vaccine-treated mice (Figure 5C and Figure S14). Additionally, an elevation in IFN-γ level was observed in the vaccinated mice (Figure S5D), which could assist in virus clearance.

The potency of the vaccine was further assessed by detecting serum levels of the antibody against the SARS-CoV-2 receptor-binding domain (RBD) that is located at the spike protein of SARS-CoV-2 and mediates the entry of viral particles into host cells with an ELISA assay kit (Figure S15). The mice in all vaccinated groups received two doses of inactivated SARS-CoV-2 vaccine, where the first dose stimulates the immune system to produce memory cells, and the second dose boosts the immune memory effects (Figure 5E,F). All the mice receiving the first dose did not elicit a significant immune response within 2 weeks, as evidenced by no visible increase in antibody levels (Figure 5G−1), consistent with previous observations. Notably, within the first week after the second dose (boosting), the antibody level began to rise in half of the mice, with an average 1.5-fold increase in the free vaccine group and a 2-fold rise in the MILD-vaccine group, in comparison with the negative control (Figure 5G−1), indicating the activation of humoral immunity. Eventually, the RBD antibody level rose to a high level (6-fold higher than the initial level) (Figure 5G−1) with an average antibody titer higher than 600 at week 6 after initial vaccination, similar to clinical trials in humans. Collectively, the MILD microneedle system effectively delivered COVID-19 vaccine in vivo for cellular and humoral immune response generation against virus. Moreover, this vaccine delivery pathway could be ideally combined with in situ vaccination counts, given dye mixture negligibly impacted vaccine stability (Figure S8), thus providing a multifunctionalized self-recording system for COVID-19 vaccination.

**Preclinical Biosafety Assessment of the MILD Microneedle-Based Vaccine Delivery System.** To evaluate the clinically translational value of the MILD microneedle-based vaccine delivery system, its biosafety was investigated comprehensively by assessment of the systemic toxicity, local damage, and recipients’ compliance. The mice receiving vaccine-loaded MILD system treatment did not indicate a obvious weight loss (Figure S16), and no histopathological alterations were observed in their main organs (i.e., liver, spleen, heart, lung, and kidneys) (Figure 6A). Moreover, the vaccine-loaded MILD system did not affect the functions of the organs as evidenced by biochemical indicators that reflect the functions of vital organs in vaccinated animals, which were comparable to those of the controls (Figure 6B and Table S1). Additionally, no detectable inflammatory cell infiltration or structural damage (Figure S17) occurred in the MILD system-treated location of the skin. Of note, the MILD systems triggered less pain, itch, redness, heat, swelling, and bleeding than the conventional hypodermic needles (Figure 6C), indicating a more favorable administration. On the basis of the above investigation, it is expected that the MILD system could act as a biocompatible, tissue-friendly, and patient-compliant alternative for vaccine delivery in clinical practice.

**DISCUSSION**

Some countries are still suffering from a sharp increase in COVID-19 confirmed cases and deaths currently, posing a high risk globally. The safe and effective vaccinations may bring great hope to help the world contain the COVID-19 pandemic by providing sufficient neutralizing antibodies to hinder virus spread. However, the heavy healthcare burden and poor patients’ compliance significantly compromised the vaccination program. Herein, in this study, we proposed and designed a smart mushroom-shaped imprintable and lightly detachable microneedle platform for the convenient and quick vaccination by providing dual functions of vaccine delivery and in situ labeling. After inserting the skin, the base layer would be readily removed, allowing the tips in the tissue for effective vaccine delivery and work as the desired pattern to indicate the vaccination information, saving a great deal of valuable time and energy for both patients and physicians, improving the current vaccination strategy.

Conventional microneedle (nondetachable) patches usually leave the patch base on the skin surface for a long time, triggering severe discomfort to the recipient as well as increasing the possibility of path loss and subcutaneous contamination. In this regard, separable microneedle patches have attracted increasing attention via base layer removal with smart design. Previously, the patch base was separated from microneedles either by bubbles weakening adhesion or by hydrophilic/hydrophobic transitions in response to temperature. In this study, the patch base could be readily separated due to the narrow concave neck intentionally designed at the interface between the tips and base layer inspired by the mushroom structure, since the contact area between the patch base and narrow neck was smaller than the area of direct contact between the base and microneedle bottom. Moreover, we also demonstrated that the concave neck did not prevent the microneedle patch from maintaining adequate mechanical strength to penetrate the skin surface. Different from other previous microneedle designs, this mushroom-inspired structure endowed the system with detachable capability in a simpler and more straightforward manner, without any stimuli but just the addition of an aqueous solution such as water, indicating an effective and biocompatible fashion.

To help vaccinators accurately record vaccination data, we proposed to encode the information in situ (on the skin) by dye-loaded MILD microneedle patches that could be arranged into the given patterns. In the ex vivo experiment (isolated porcine skin), we selected crystal violet-loaded MILD patches to organize the patterns that could be identified under both natural light and fluorescence excitation. In a rat model, a more biocompatible FITC dye was applied, consistent with in vitro labeling, suggesting a general labeling strategy. Considering the water solubility and quick diffusion of these dyes, a small amount of PCL was selected as a slow-degrading polyester to retard the dispersion of the dyes, extending the labeling period. Notably, for both isolated porcine skin and living objects, the MILD microneedle patterns displayed clearly visible signals in a dotted matrix for quite a long time (at least 28 days longer than the most recommended vaccination intervals) without any obvious decay or photobleaching, demonstrating excellent photostability. During this period, the vaccine protein amount in microneedles exhibited negligible alteration at room temperature, indicating a long-term storage.
stability of the vaccine in the MILD microneedle system, consistent with the previous study. Moreover, appropriate dye/PCL ratio optimization can further prolong the in situ imprinting time by MILD microneedle patterns, which can meet even longer vaccination interval requirements if necessary. It should be noted that the FITC dye imaging may require excitation by the light source at specific wavelengths, which may still pose an obstacle for the general application of the system. Fortunately, a smartphone-based imaging system that combines the external optical systems with mobile phones via wired/wireless communication has been well developed, which would be integrated into our MILD system at the next step. With the help of these intelligent technologies, this MILD microneedle platform is believed to be readily self-administered by an individual, suggesting highly clinical and commercial values.

Currently, COVID-19 vaccines have been tested as an efficient strategy to prevent people from serious illness or death caused by SARS-CoV-2 infection, but most of them required multiple doses to build complete immune protection. Nevertheless, a heavy healthcare burden accompanied by numerous operations of vaccination data storage and access may result in some inevitable mistakes to mislead doctors, posing a high risk to vaccinators. In this study, we proposed to record information in situ by arranging different MILD microneedle patterns in a visualized way. For example, MILD microneedle patches were organized into Arabic numerals to record the counts of vaccine doses directly on administrative site, avoiding data storage in specific equipment, saving a great deal of time and energy for vaccinators and physicians.

The efficiency of the MILD system for vaccine delivery was tested comprehensively in the rodent models. Due to the limited vaccines that we obtained for our study, we did not conduct large-scale vaccine delivery trials in large animals or humans currently, but its potential for safe and effective COVID-19 vaccination was confirmed by the evidence that the mice receiving two doses of MILD-vaccine generated effective cellular and humoral immunity, as well as high-level RBD antibodies with no significant systemic toxicity or local skin damage and infection. In comparison with the conventional solution-based vaccine, the MILD platform presented superior advantages in improving people’s compliance and quick in situ data storage, and therefore may serve as a general strategy for large-scale vaccination globally.

**CONCLUSION**

To combat the vaccination challenges caused by the current COVID-19 pandemic, we developed a MILD microneedle-based platform for the effortless self-administration of the COVID-19 vaccine and data record by providing painless penetration and in situ labeling. In this MILD platform, the patch base could be readily removed from the skin surface to improve recipients’ comfort and facilitate vaccine delivery. Besides, dye-integration endows the MILD system with quick recording and tracking of vaccination information after building block arrangement, which is especially critical for vaccines that require multiple doses. Overall, we believe this smart bioinspired MILD platform may serve as a promising candidate to fight against the current COVID-19 pandemic, and may also act as a general strategy for other diseases that require large-scale vaccinations.

**METHODS**

**Fabrication of MILD Microneedle Patches.** The master molds were designed with the Solidworks 2020 software and printed in an X190 3D printer (Xiaoyanger, China) with orange resin. PDMS mold (negative) was fabricated from the master mold by mixing two-part resin systems containing vinyl groups (part A) and hydroxiloxane groups (part B) at the ratio of 10:1 (Sylgard 184 (Dow, Midland, MI, USA)), 150 μL of inactivated SARS-CoV-2 vaccine (around 60 μg/mL) from Wuhhan Institute of Biological Products Co., Ltd., and Wuhhan Institute of Virology, Chinese Academy of Sciences (CAS) was then added into the mold and vacuumed (2.5 kPa) for 10 min. After centrifugation (2000 rpm, 10 min), 250 μL of 1.5% ALG (Macklin, China) and 250 μL of 1.5% CMC (Aladdin, China) were mixed and introduced into the mold. After another centrifugation (2000 rpm, 10 min), 1 mL of 1.5% ALG and 1 mL of 1.5% CMC were mixed and cast into the mold with 100 μL of 0.1 mg/mL crystal violet (Beyotime, China) or 0.1 mg/mL FITC (Merck, Germany) dye solution. The mold was then centrifuged (2000 rpm, 10 min) and placed under 37 °C overnight. 20 μL of 10% PCL (ShanghaiHai YuanYe Bio., China) was dissolved in the dichloromethane, and integrated in the system. To fabricate the base layer, 1 mL of 3% CMC was applied, which would build a thin support after drying, and the MILD microneedle patch was carefully removed from the mold. A dip-coating method was applied to enhance the mechanical strength of the patch by using 1 M CaCl₂ solution, and the vaccine-loaded MILD microneedle patches were stored at 4 °C. In different microneedle patterns, the vaccine was loaded in only one block.

**Characterization of MILD Microneedle Patches.** The shape and surface morphology of MILD patches were directly observed under an MZ62−3D microscope (Mshot, China) and an SEM (TESCAN VEGA3, China), respectively. The mechanical strength of the microneedle patches was tested with an MTS 30 G tensile testing machine according to a previously reported method. The loading efficiency, vaccine stability and release behavior were tested through the detection of protein concentrations by BCA Protein Assay Kit (Beyotime, China) according to the manufacturer’s instructions.

**Ex Vivo Insertion Test.** To evaluate the imprinting capability of the MILD system, 1.4% agarose gel was applied to mimic the skin strength. Briefly, 1.4 g of agarose (BioFroux, Germany) was dissolved into 100 mL of deionized water, followed by heating in a microwave oven until the mixed solution turned transparent. The solution was then poured into a 60 mm Petri dish and cooled at room temperature. The MILD microneedle patches were inserted into the gel and then treated with deionized water (200 μL per patch). The base layer was carefully peeled by hand, and needle tips were retained in the gel, indicating successful imprint. Moreover, the similar treatment was tested on the porcine skin, consistent with agarose gel test.

**In Situ Imprint for Vaccine Counts.** Aiming to record the vaccine information, the MILD microneedle patches were organized to pattern “1”, “2”, or “3” to achieve in situ labeling. 0.01 mg crystal violet was encapsulated into the MILD system as the indicators, which could be excited at a wavelength of 620 nm to emit signals at 700 nm. After imprint, the pattern in the porcine skin could be directly detected under the In Vivo imaging system (BRUKER In-Vivo FX PRO, USA). The exposure time ranges from 1 to 3 s, f-stop was set as 0.95.

Moreover, this imprint effect was investigated in Sprague–Dawley (SD) rats (6 weeks old, purchased from SipieFu (Beijing) Biotechnology Co., Ltd., China). Different microneedle patterns were inserted into the dorsal of rats followed by base removal with sterile saline solution (0.5 mL) and careful peeling. With the help of the medical tape (2 cm × 6 cm), the MILD microneedle patches were firmly attached to the dorsal area for up to 28 days, which contained 0.01 mg FITC in each patch. Each sample (pattern “1”, pattern “2” and pattern “3” containing 3 rats) was observed under a BRUKER In-Vivo FX PRO in Vivo imaging system at given time points (day 1, day 14 and day 28). The exposure time ranges from 1 to 2 s, f-stop was set as 0.95. The excitation wavelength was set at 490 nm, and emission at
Supplementary figures: images of the MILD microneedle patches before and after loading SARS-CoV-2 vaccines; long-term storage of vaccines in the MILD microneedle patch; dimension control for MILD microneedle patches; comparison of mechanical strength of the MILD microneedle patches with different components; images presenting the water solubility of different MILD microneedle patches; release profile of vaccine from the MILD microneedle patch; removal of microneedle base by tensile force and water dissolving; stability of SARS-CoV-2 vaccines after being mixed with different dyes; HE staining image of the porcine skin after being inserted by the MILD microneedle; fluorescence images of rats on day 1, 14, and 28 after MILD microneedle pattern imprint; the fluorescence distribution of a single microneedle patch embedded in the dorsal of the rat; microneedle pattern design; images of mice inserted with a vaccine-loaded MILD microneedle patch; corresponding quantification of immune cells in LNs and spleen; schematic diagram presenting the detection of SARS-CoV-2 RBD antibody by indirect ELISA assay; the body weight changing curves of the mice in each group; HE staining images of the skin isolated from the mice at week 8 after initial treatment with PBS or MILD-vaccine; Supplementary table: biochemical analysis in the blood sera of each mouse with the given treatment (PDF)

ASSOCIATED CONTENT

* Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsnano.1c10718.

530 nm was detected. All data are quantitated by using Bruker MI SE 721 software.

**COVID-19 Vaccination and RBD Antibody Detection.** Female BALB/c mice (6 weeks old, purchased from SiPeiFu (Beijing) Biotechnology Co., Ltd., China) were randomly divided into three groups (4 mice in each group) and their right infra-axillary dermis was treated with 150 μL of PBS (injection), 150 μL of free inactivated SARS-CoV-2 vaccine (around 60 μg/mL, injection) or MILD system containing 150 μL vaccine (patch). Two doses were conducted with a time interval of 14 days. Blood samples (100 μL per mouse) were collected from the retro-orbital plexus of mice with sterilized capillary tubes every week. The sera were then isolated by centrifuge and detected with a SARS-CoV-2 RBD Mouse Antibody Assay Kit (ELISA) (AbMax Biotechnology Co., Ltd., China). The OD values were read using a microplate reader (Tecan Infinite F50, Switzerland), and the results were expressed as the relative OD value compared with the control group (PBS treatment). To analyze lymphocyte response and cytokines, mice were treated with 150 μL of PBS, 150 μL of free inactivated SARS-CoV-2 vaccine, or MILD system containing 150 μL vaccine for one-dose. On day 24, LNs, spleens, and blood were collected from mice in each group. LNs and spleens were pulverized, and the collected cells were washed with PBS followed by staining with specific antibodies including CD45-APC/Cyamine7, CD3-FTTC, CD8a- Brilliant Violet 510, IFN-γ-PE, IL-2- Brilliant Violet 421, CD19-PE/Cyamine7 and CD27-APC (BioLegend, USA). The cell samples were then analyzed by flow cytometry (Canto II, BD Company, USA). Quantification of IFN-γ levels in isolated sera was performed using an IFN-γ precoated ELISA kit (Ruixin Biotech, China) following the manufacturer’s protocol.

**Systemic Biosafety Evaluation after MILD Microneedle Treatment.** Blood samples were obtained from the retro-orbital plexus of mice 8 weeks after the initial dose. Sera were centrifugally collected and analyzed with a clinical chemistry analyzer (Beckman Coulter, USA) for cardiac, hepatic, and renal function evaluation. Livers, hearts, lungs, spleens, kidneys, and skins treated with conventional hypodermic needles or MILD microneedles were isolated from mice in each group and then fixed with 4% paraformaldehyde, embedded in paraffin, and stained with hematoxylin and eosin after being sliced (15 μm). The stained tissue sections were then observed under an inverted microscope (Olympus IX71, Japan) equipped with a DP73 digital camera. All the animal experiments described above were approved by the institutional animal care and use committee, Huazhong University of Science and Technology, Wuhan, China.

**Volunteer Test.** In order to evaluate the recipients’ preference on the MILD microneedle-based vaccination operation, we compared people’s skin reactions after a conventional hypodermic needle-based injection and MILD system treatment. A questionnaire survey was completed by six volunteers that were treated with a hypodermic needle or the MILD system. Skin reactions including redness, swelling, heat, pain, itching, and bleeding were recorded and scored (score 4, very serious; score 3, serious; score 2, mild reaction; score 1, no reaction). The experiments were authorized by the Ethics Committee of Wuhan Union Hospital and the ethics committee of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China.

**Statistical Analysis.** All experiments were repeated at least three times and the data were presented as mean ± standard deviation (SD). Student’s t test was performed when two independent sets of data were presented. One-way ANOVA with post hoc tests were employed for comparative analysis of more than two independent groups. Statistical significance was indicated by asterisks (*p < 0.05, **p < 0.01, ***p < 0.001), and N.S. suggested no significant difference.

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L.W., Z.W., W.C., and X.C. proposed the study concept. Q.L., R.X., H.F., and W.C. designed and conducted the experiments, interpreted the data, and drafted the manuscript. J.X., Y.X., P.C., Y.Z., and T.L. assisted in the experiment conduction. L.W., Z.W., W.C., and X.C. supervised the study, reviewed the data, and revised the manuscript.

Notes
The authors declare no competing financial interest.

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REFERENCES
(1) Bundgaard, H.; Bundgaard, J. S.; Raaschou-Pedersen, D. E. T.; von Buchwald, C.; Tønnesen, T.; Norskov, J. L.; Pries-Heje, M. M.; Norrild, C. R.; Nielsen, P. B.; Winslow, U. C.; Fogh, K.; Hasselbalch, R.; Sørensen, L. J. H.; Ringgaard, A.; Porsborg, Anders; M.; Goecke, N. B.; Trehub, H.; Skovgaard, K.; Benfield, T.; Ullum, H.; Torp-Pedersen, C.; Iversen, L. K. Effectiveness of Adding a Mask Recommendation to Other Public Health Measures to Prevent SARS-CoV-2 Infection in Danish Mask Wearers: A Randomized Controlled Trial. Ann. Int. Med. 2021, 174 (3), 335–343.
(2) Ferra, S. N. Non-pharmaceutical Interventions During the COVID-19 Pandemic: A Review. Phys. Rep. 2021, 913, 1–52.
(3) Jeyanathan, M.; Akhrami, S.; Small, F.; Miller, M. S.; Lichby, B. D.; Xing, Z. Immunological Considerations for COVID-19 Vaccine Strategies. Nat. Rev. Immunol. 2020, 20 (10), 615–632.
(4) Shin, M. D.; Shukla, S.; Chung, Y. H.; Beiss, V.; Chan, S. K.; Ortega-Rivera, O. A.; Wirth, D. M.; Chen, A.; Sack, M.; Pokorski, J. K.; Steinmetz, N. F. COVID-19 Vaccine Development and a Potential Nanomaterial Path Forward. Nat. Nanotechnol. 2020, 15 (8), 646–655.
(5) Sheng, T.; Luo, B.; Zhang, W.; Ge, X.; Yu, J.; Zhang, Y.; Gu, Z. Microneedle-Mediated Vaccination: Innovation and Translation. Adv. Drug Delivery Rev. 2021, 179, 113919.
(6) Park, K. S.; Sun, X.; Aikins, M. E.; Moon, J. J. Non-viral COVID-19 Vaccine Delivery Systems. Adv. Drug Delivery Rev. 2021, 169, 137–151.
(7) Ahmed, S.; Khan, S.; Imam, I.; Al Mughairbi, F.; Sheikh, F. S.; Hussain, J.; Khan, A.; Al-Harrasi, A. Vaccine Development against COVID-19: Study from Pre-Clinical Phases to Clinical Trials and Global Use. Vaccines (Basel) 2021, 9 (8), 836.
(8) Al Kaabi, N.; Zhang, Y.; Xia, S.; Yang, Y.; Al Qahtani, M. M.; Abdulrazzaq, N. M.; Al Nusairi, M.; Hassany, M.; Jawad, J. S.; Abidalla, J.; Hussein, S. E.; Al Mazrouei, S. K.; Al Karam, M.; Li, X.; Yang, X.; Wang, W.; Lai, B.; Chen, W.; Huang, S.; Wang, Q.; Yang, T.; Liu, Y.; Ma, R.; Hussain, Z. M.; Khan, T.; Safiuddin Fasihuddin, M.; You, W.; Xie, Z.; Zhao, Y.; Jiang, Z.; Zhao, G.; Zhang, Y.; Mahmoud, S.; El-Fantawy, I.; Xiao, P.; Koshy, A.; Zaher, A.; Wu, H.; Duan, K.; Pan, A.; Yang, X. Effect of 2 Inactivated SARS-CoV-2 Vaccines on Symptomatic COVID-19 Infection in Adults: A Randomized Clinical Trial. JAMA 2021, 326 (1), 35–45.
(9) Moss, W. J.; Gostin, L. O.; Nuzzo, J. B. Pediatric COVID-19 Vaccines: What Parents, Practitioners, and Policy Makers Need to Know. JAMA 2021, 326, 20734.
(10) Chen, W.; Wang, Z.; Wang, L.; Chen, X. Smart Chemical Engineering-based Lightweight and Miniaturized Attachable Systems for Advanced Drug Delivery and Diagnostics. Adv. Mater. 2022, 34, 2106701.
(11) Chen, W.; Cai, B.; Geng, Z.; Chen, F.; Wang, Z.; Wang, L.; Chen, X. Reducing False Negatives in COVID-19 Testing by Using Microneedle-Based Oropharyngeal Swabs. Matter-Uris 2020, 3 (5), 1589–1600.

(12) Chen, W.; Tian, R.; Xu, C.; Yung, B. C.; Wang, G.; Liu, Y.; Ni, Q.; Zhang, F.; Zhou, Z.; Wang, J.; Niou, G.; Ma, Y.; Fu, L.; Chen, X. Microneedle-array Patches Loaded with Dual Mineralized Protein/Peptide Particles for Type 2 Diabetes Therapy. Nat. Commun. 2017, 8 (1), 1777.

(13) Sanjay, S. T.; Zhou, W.; Dou, M.; Takavoli, H.; Ma, L.; Xu, F.; Li, X. Recent Advances of Controlled Drug Delivery Using Microfluidic Platforms. Adv. Drug Deliver. Rev. 2018, 128, 3–28.

(14) Bae, W. G.; Ko, H.; So, J. Y.; Yi, H.; Lee, C. H.; Lee, D. H.; Ahn, Y.; Lee, S. H.; Lee, K.; Jun, J.; Kim, H. H.; Jeon, N. L.; Jung, W.; Song, C. S.; Kim, T.; Kim, Y. C.; Jeong, H. E. Snake Fang-Inspired Stamping Patch for Transdermal Delivery Of Liquid Formulations. Sci. Transl. Med. 2019, 11 (503), No. eaaw3329.

(15) Demuth, P. C.; Garcia-Beltran, W. F.; Ai-Ling, M. L.; Hammond, P. T.; Irvine, D. J. Composite Dissolving Microneedles for Coordinated Control of Antigen and Adjutant Delivery Kinetics in Transcutaneous Vaccination. Adv. Funct. Mater. 2013, 23 (2), 161–172.

(16) Jamaledin, R.; Yiu, C. K. Y.; Zare, E. N.; Niu, L. N.; Vecchione, R.; Chen, G.; Gu, Z.; Tay, F. R.; Makvandi, P. Advances in Antimicrobial Microneedle Patches for Combating Infections. Adv. Mater. 2020, 32 (33), No. e2002129.

(17) Tran, K. T. M.; Gavitt, T. D.; Farrell, N. J.; Curry, E. J.; Mara, A. B.; Patel, A.; Brown, L.; Kilpatrick, S.; Piotrowska, R.; Mishra, N.; Szczepanek, S. M.; Nguyen, T. D. Transdermal Microneedles for the Programmable Burst Release of Multiple Vaccine Payloads. Nat. Biomed. Eng. 2021, 5 (9), 998–1007.

(18) Poirier, D.; Renaud, F.; Dewar, V.; Strodiot, L.; Wauters, F.; Janimak, J.; Shimada, T.; Nomura, T.; Kabata, K.; Kuruma, K.; Kasuno, T.; Sakai, M.; Nagasaki, H.; Oyamada, T. Hepatitis B Surface Antigen Incorporated in Dissolvable Microneedle Array Patch is Antigenic and Thermostable. Biomaterials 2017, 145, 256–265.

(19) Uppu, D.; Turvey, M. E.; Sharif, A. R. M.; Bidet, K.; He, Y.; Ho, V.; Tambe, A. D.; Lescar, J.; Tan, E. Y.; Fink, K.; Chen, J.; Hammond, P. T. Temporal Release of a Three-Component Protein Subunit Vaccine from Polymer Multilayers. J. Controlled Release 2020, 317, 130–141.

(20) Kim, Y. C.; Park, J. H.; Prausnitz, M. R. Microneedles for Drug and Vaccine Delivery. Adv. Drug Deliver. Rev. 2012, 64 (14), 1547-1563.

(21) Korkmaz, E.; Balment, S. C.; Sumpter, T. L.; Carey, C. D.; Erdos, G.; Falo, L. D., Jr. Microarray Patches Enable the Development of Skin-Targeted Vaccines against COVID-19. Adv. Drug Deliver Rev. 2021, 171, 164–186.

(22) Liu, M.; Li, J.; Zhou, X.; Li, J.; Feng, S.; Cheng, Y.; Wang, S.; Wang, Z. Inhibiting Random Droplet Motion on Hot Surfaces by Engineering Symmetry-Breaking Janus-Mushroom Structure. Adv. Mater. 2020, 32 (14), No. e1907999.

(23) Yi, H.; Kang, M.; Kwak, M. K.; Jeong, H. E. Simple and Reliable Fabrication of Bioinspired Mushroom-Shaped Micropillars with Precisely Controlled Tip Geometries. ACS Appl. Mater. Interfaces 2016, 8 (34), 22671–22678.

(24) Kim, J. D.; Kim, M.; Yang, H.; Lee, K.; Jung, H. Droplet-born Air Blowing: Novel Dissolving Microneedle Fabrication. J. Controlled Release 2013, 170 (3), 430–436.

(25) Irvine, D. J.; Hanson, M. C.; Rakha, K.; Tokatlian, T. Synthetic Nanoparticles for Vaccines and Immunotherapy. Chem. Rev. 2015, 115 (19), 11109–11146.

(26) Malkinmammadov, E.; Tanir, T. E.; Kiziltay, A.; Hasirci, V.; Hasirci, N. PCL and PCL-based Materials in Biomedical Applications. J. Biomater. Sci. Polym. Ed 2018, 29 (7–9), 863–893.

(27) He, X. H.; Hu, L. Q.; Zhou, L. S.; Zhong, J. C.; Luo, H.; Pu, Z. J. Enhanced Fluorescence Properties of Flexible Waterborne Polyurethane Films by Blocking Fluorescein Isothiocyanate (FITC). Mater. Lett. 2021, 293, 129668.
CorV: a Randomised, Double-Blind, Placebo-Controlled, Phase 1/2 Trial. Lancet Infect. Dis. 2021, 21 (1), 39–51.

(38) Ortega-Rivera, O. A.; Shin, M. D.; Chen, A.; Beiss, V.; Moreno-Gonzalez, M. A.; Lopez-Ramirez, M. A.; Reynoso, M.; Wang, H.; Hurst, B. L.; Wang, J.; Pokorski, J. K.; Steinmetz, N. F. Trivalent Subunit Vaccine Candidates for COVID-19 and Their Delivery Devices. J. Am. Chem. Soc. 2021, 143 (36), 14748–14765.

(39) Li, W.; Tang, J.; Terry, R. N.; Li, S.; Brunie, A.; Callahan, R. L.; Noel, R. K.; Rodriguez, C. A.; Schwendeman, S. P.; Prausnitz, M. R. Long-acting Reversible Contraception by Effervescent Microneedle Patch. Sci. Adv. 2019, 5 (11), eaaw8145.

(40) Li, W.; Terry, R. N.; Tang, J.; Feng, M. R.; Schwendeman, S. P.; Prausnitz, M. R. Rapidly Separable Microneedle Patch for the Sustained Release of a Contraceptive. Nat. Biomed. Eng. 2019, 3 (3), 220–229.

(41) Vasconcellos, L. M. R.; Elias, C. M. V.; Minhoto, G. B.; Abdala, J. M. A.; Andrade, T. M.; de Araujo, J. C. R.; Gusmao, S. B. S.; Viana, B. C.; Marciano, F. R.; Lobo, A. O. Rotary-jet Spun Polycaprolactone/Nano-Hydroxyapatite Scaffolds Modified by Simulated Body Fluid Influenced the Flexural Mode of the Neoformed Bone. J. Mater. Sci. Mater. Med. 2020, 31 (8), 72.

(42) Leroux, A.; Venkatesan, J. K.; Castner, D. G.; Cucchiarini, M.; Mignonney, V. Analysis of Early Cellular Responses of Anterior Cruciate Ligament Fibroblasts Seeded on Different Molecular Weight Polycaprolactone Films Functionalized by a Bioactive poly(sodium styrene sulfonate) polymer. Biohyperphases 2019, 14 (4), 041004.

(43) World Health Organization. COVID-19 vaccine tracker and landscape. https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines (accessed on February 22, 2022).

(44) McHugh, K. J.; Jing, L.; Severt, S. Y.; Cruz, M.; Sarmadi, M.; Jayawardena, H. S. N.; Perkinsson, C. F.; Larusson, F.; Rose, S.; Tomasic, S.; Graf, T.; Tzeng, S. Y.; Sugarman, J. L.; Vlasic, D.; Peters, M.; Peterson, N.; Wood, L.; Tang, W.; Yeom, J.; Collins, J.; Welkoff, P. A.; Karchin, A.; Tse, M.; Gao, M.; Bawendi, M. G.; Langer, R.; Jaklenec, A. Biocompatible Near-Infrared Quantum Dots Delivered to the Skin by Microneedle Patches Record Vaccination. Sci. Transl. Med. 2019, 11 (523), No. eaay7162.

(45) Demchenko, A. P. Photobleaching of Organic Fluorophores: Quantitative Characterization, Mechanisms, Protection. Methods Appl. Fluoresc. 2020, 8 (2), 022001.

(46) Hunt, B.; Ruiz, A.; Pogue, B. Smartphone-based Imaging Systems for Medical Applications: a Critical Review. J. Biomed. Opt. 2021, 26 (4), 040902.

(47) Prausnitz, M. R. Engineering Microneedle Patches for Vaccination and Drug Delivery to Skin. Annu. Rev. Chem. Biomol. Eng. 2017, 8, 177–200.