TAILORED FOREST MICRONEEDLES USING CROSS OVER LINES LASER LITHOGRAPHY FOR SIMULTANEOUS DELIVERY OF MULTIPLE DRUGS

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ABSTRACT
This paper explores a low-cost and scalable method called Cross-Over-Lines (COL) laser lithography to create multi-geometry microneedles on a single patch. By adjusting laser settings, we can control the size and shape of conical microneedles. This opens up exciting possibilities of incorporating multiple drugs within a single patch, with each drug housed in microneedles of a specific size for optimal delivery. As a proof of concept, we successfully loaded and delivered two drugs simultaneously: Simvastatin (anti-inflammatory) and Tetracycline Hydrochloride (antimicrobial) to stimulate wound healing. This innovation could create unique drug release profiles and potentially treat conditions requiring multiple medications.

KEYWORDS
COL laser lithography, laser parameters, multi-height microneedles, wound healing, Simvastatin and Tetracycline Hydrochloride

INTRODUCTION
Microneedles are emerging as a robust way to deliver therapeutic moieties through a painless and easy-to-use transdermal patch. Although there are numerous approaches to fabricate microneedles, direct write approach using cross-over-lines (COL) laser lithography using commercially available CO_2 lasers offers the advantage of being low-cost and a scalable technology.

The COL method utilizes a three-step cleanroom-free method to make microneedles molds [1]. A specific two-dimensional (2D) geometric pattern consisting of lines that cross over each other at one point to ablate a sharp conical shape in an acrylic sheet enables creation of conical microneedle molds (**Figure 1(a)**). Tuning laser parameter enables fabrication of microneedle molds with different dimensions for transdermal drug delivery [2,3]. In this article, we have utilized this tunability to enable fabrication of microneedles of different geometries and heights on one single patch by exploring different laser cutting parameters including laser power, speed and focus distance. Studies have shown a link between microneedle shape and how well it delivers drugs [4].

By fine-tuning the laser settings, we can fabricate microneedles in customized heights and sizes, allowing to incorporate multiple drugs within the same patch. Each drug can be strategically loaded into microneedles specifically designed for optimal delivery based on its properties [5]. For example, a drug needing to reach deeper skin layers could be housed in taller microneedles, while another medication targeting the upper layers could reside in shorter microneedles. This precise control over microneedle dimensions allows us to not only control the delivery depth of each drug but also its spatial distribution within the skin. By selecting which microneedles receive specific drug formulations, we can create customized patterns on the skin. As a proof of concept, we have demonstrated the loading and simultaneous delivery of two drugs – Simvastatin (anti-inflammatory) and Tetracycline Hydrochloride (TH) (antimicrobial) for promoting wound healing [5].

This platform unlocks the creation of personalized patches in any configuration, enabling targeted delivery of various drugs at specific depths within the skin with a tunable delivery profile. Especially in burn wounds where the burn area is non-uniform, a patch matching the shape of the wound area can be fabricated for maximum efficacy. To the best of our knowledge, this is the first platform to have delivered multiple drugs using a single microneedle patch fabricated with any laser-based approach.

MATERIALS AND METHODS
The negative microneedle mold was ablated by a CO_2 laser onto a 6 mm thick piece of polymethyl methacrylate (PMMA). The laser traces the 4-cross design (**Figure 1(a)**) in defined speed, power, and laser focus combination to get microneedles of desired height and shape. The process to make microneedles molds is demonstrated in **Figure 1(a)**.

To enable loading of multiple drugs, the specific areas of the PMMA mold were rastered creating individual wells for loading different drugs without any mixing as shown in **Figure 1(b)**. To make the dual drug loaded patch, 10 mg of each drug was separately added to 10mg/mL of zein solution (made in 80% ethanol) and loaded onto the PDMS negative microneedle molds.

Microneedle mechanical strength was evaluated under axial load to ensure stability during insertion. The release profiles of both drugs were assessed in vitro using phosphate-buffered saline (PBS). Additionally, the penetration capabilities of the multi-height microneedles were investigated using pig skin.

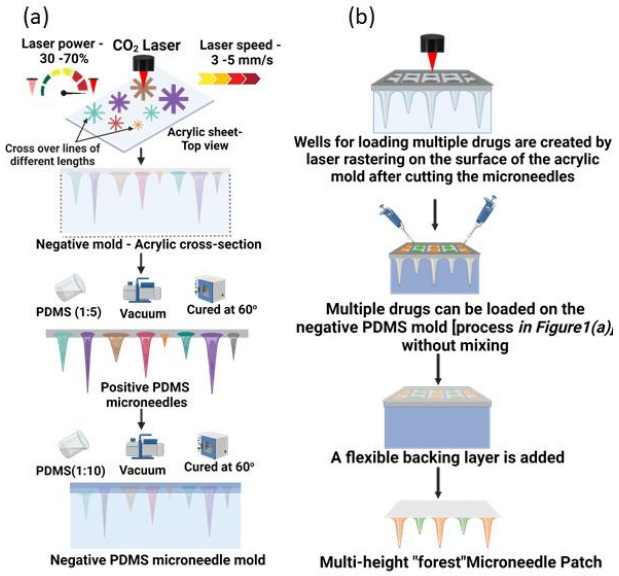

Figure 1: Fabrication work flow for micronedles (a) Schematic to illustrate the microneedle fabrication using COL laser lithography. (b)Schematic of the laser rastering process to load multiple drugs.

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RESULTS AND DISSCUSSION

Multi-geometry microneedles:

Previous research has found the correlation between microneedle geometry and therapeutic delivery profile for conical microneedles. Height, base diameter, tip diameter, tip angle, and needle shape are the factors that define the geometry of a conical microneedle. However, not all these factors are weighted equally when it comes to microneedle efficacy. Height is viewed as one of the most important factors in microneedle geometry. Maximizing the height of the microneedle increases the depth at which the drug can reach, but it also increases the chance that the patient will feel pain upon administration. Base diameter is also an important design consideration. A combination of the longest needle length and smallest diameter had the deepest penetration regardless of tip diameter. Conversely, the shortest needle length and largest base diameter had the least penetration. This suggests that base diameter influences the penetration ability more than the tip diameter [4].

The CO_2 laser allowed us to input values for speed, power, and laser focus. We utilized these three parameters to achieve different needle geometries. These parameters can vary between each needle, but they can also vary between each cut on one individual needle.

The speed and power of the laser can be assigned separately as a value between 0 and 100—with 0 being 0% power and 100 being 100% power. The speed was varied between 3 to 5 mm/s. Both the speed and the power of the laser impacts the depth to which the laser can reach. When a slower speed or higher power is used, it results in a deeper cut.

Other laser parameters that effect the microneedle geometry are the laser focus and the number of repeat cuts in the design. The focus distance is the distance between the laser tip and the surface to be etched. Changing the focus distance impacts the sharpness of the needle tip. This fine-tuning allows for intentional over-focusing or under-focusing of the laser beam relative to the acrylic sheet. Over-focusing creates microneedles with sharper tips, while under-focusing results in lesser sharp tips but with a smoother microneedle base allowing for better adhesion of the patch onto the skin. This control over laser focus provides an additional dimension for tailoring geometry of the microneedles.

To demonstrate the flexibility of this technology, we have fabricated a patch having microneedles of different heights (**Figure 1(a)**), and a patch with base diameters (**Figure 1(b)**). We also fabricated microneedles in the letters "NANOLAB" (**Figure 1(c-d)**). There were eleven different needle designs on the NANOLAB microneedle patch. Each design varied in diameter, height, and tip sharpness. The resulting microneedles consists of different heights, widths and spacing result in a forest configuration, hence the term "Forest microneedles. A multi-regression model to map the effect on the multi-independent laser parameters on dependent variables like the needle height, spacing, roughness, and tip diameter has been investigated for the first time (data not shown).

Multi-height microneedles for simultaneous drug delivery

This study explored the potential of a microneedle patch for simultaneous delivery of two drugs: simvastatin (anti-inflammatory) and tetracycline hydrochloride (antibacterial). Simvastatin and tetracycline hydrochloride were chosen for this study because of their respective properties in treating wounds [5, 6,7].

With all other microneedle parameters held constant, the design incorporated needles of varying heights: 1100 µm for simvastatin and 670 µm for tetracycline hydrochloride as shown in **Figure 3.**

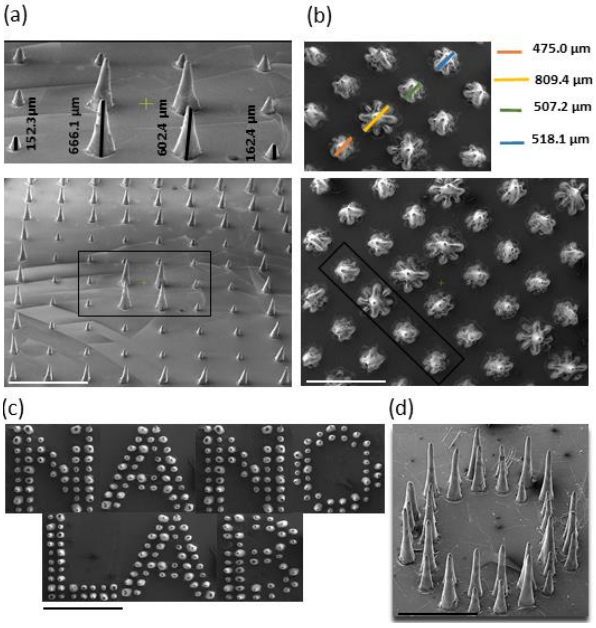

Figure 2: SEM images of the multi-geometry PDMS microneedles. (a) with different heights (Scale bar = 1 mm) (c) with different base diameters (Scale bar = 1mm) (d) Top view of microneedle fabricated in the letters "NANOLAB" (scale – 400 µm). (d) Angled view of the letter "O" showing the different heights (Scale – 350 µm).

This strategic design allows for deeper penetration of simvastatin, potentially targeting nerves in the dermis to reduce inflammation. Conversely, the shorter tetracycline needles primarily target the wound surface, effectively preventing infection by combating bacteria. A flexible backing layer allows for better conformation onto the skin. The dual drug loaded microneedle patch with all the notable dimensions are show in **Figure 3(a-c)**.

Mechanical characterization

This insertion force depends on several factors, including the microneedle's shape, aspect ratio tip diameter, and spacing between needles. Since polymer microneedles are inherently weaker than their metal counterparts (due to a lower Young's modulus), they often require a wider base for added stability during insertion.

The aspect ratio for the simvastatin and TH microneedles are 2.75 and 1.64 respectively. Prior research has shown that a force of 5 N is needed for effective insertion of polyvinyl alcohol microneedles into pig skin [8]. In contrast, microneedles with zein as a base polymer require a significantly lower force per needle (0.4 N), as also demonstrated in **Figure 4** with the force-displacement graph. Incorporating glycerol into zein has further enhanced its mechanical properties, making it an even stronger polymer for microneedle fabrication [9].

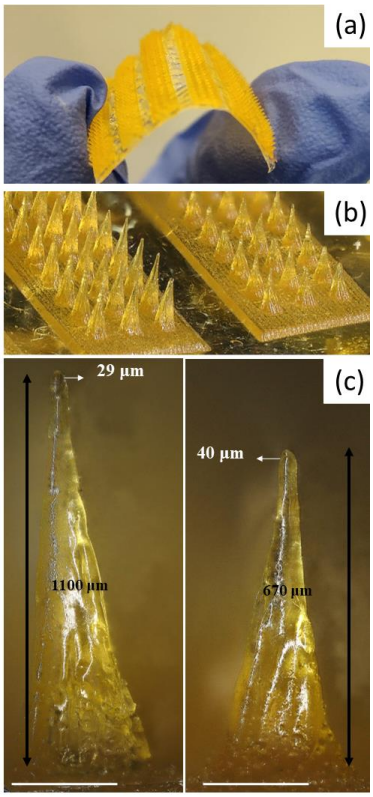

Figure 3(a) Microneedle patch with flexible backing layer for better conformability. (c) Image of the dual drug loaded patch taken with an optical microscope showing distinct microneedles strips for the two drugs (Scale bar = 1 mm). (c) Individual microneedles with the microneedle height and tip diameter. Simvastatin is loaded in the taller microneedle while TH is loaded in the short microneedle.

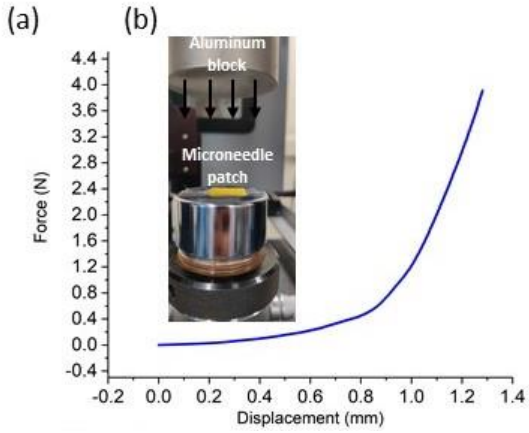

Figure 4(a) Mechanical behavior of the microneedle patches under normal compressive load and (b) Image of the experimental setup (inset).

Drug delivery profile

The delivery of both the drugs were conducted invitro with phosphate buffer saline (PBS) at 37 degrees. PBS was collected as at different time points and Simvastatin and TH in the aliquots was quantified with UV-VIS spectroscopy at 238 and 287 nm respectively. The release was studied over 4 days as demonstrated in **Figure 5**.

The procedure used to quantify the drug delivery profile of both simvastatin and tetracycline hydrochloride provided evidence that differing needle geometries led to different therapeutic delivery profiles for the same drug concentration. The simvastatin needles (1100 ± 30 µm) were fabricated at a longer length than the tetracycline hydrocholoride needles (670 ± 45 µm)—thus resulting in a larger surface area. With a larger surface area comes a greater amount of drug release, which explains the larger percentage of simvastatin release compared to tetracycline hydrocholoride in the drug release study.

Simvastatin stimulates the secretion of vascular endothelial growth factor (VEGF). VEGF, regulating blood vessel production, serves as an indicator of wound healing when present in wounds. Evidence suggests that Simvastatin accelerates wound healing by promoting both angiogenesis and lymph angiogenesis. Angiogenesis enhances wound vascularity, a crucial factor in promoting healing, particularly in cutaneous wound healing. Applying simvastatin accelerates the growth of granulation tissues by facilitating inflammation, collagen deposition, and re-epithelialization. Furthermore, Simvastatin enhances the presence of macrophages in the wound, which play a pivotal role in initiating wound healing by enhancing proinflammatory functions and stimulating the growth of fibroblasts, keratinocytes, and endothelial cells [6,11].

Tetracycline is selected as antimicrobial because it is a widely used antibiotic to treat infections both Gram-positive and Gram-negative strains. Recently, Zhao et al. compared microneedle delivery of tetracycline hydrochloride (640mg/kg) in rats to a lower, intravenous dose (50mg/kg). They found microneedles delivered more drug over time (AUC: 82.03 µg*h/ml* vs. 33.56 µgh/ml) despite a lower peak concentration (7.40 µg/ml vs. 8.86 µg/ml). This suggests microneedles achieved a sustained-release effect [5].

Combined, these two drugs provide antimicrobial and anti-inflammatory properties, offering a promising strategy for wound healing applications.

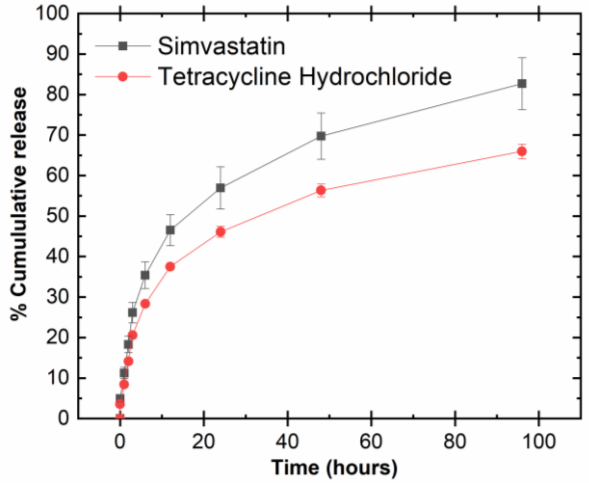

Figure 5: Drug delivery profile of Simvastatin and TH in PBS over the course of 4 days. Both the drugs were loaded on one patch.

Microneedle penetration studies

The dual drug loaded patch successfully penetrated pig skin as seen by the puncture wounds in **Figure 6(a)**. The efficiency of the microneedle penetration was calculated for the tall and the short needles separately. The tall (simvastatin loaded) microneedles had a

penetration efficiency of 92% while the short (TH loaded) microneedles had an 87% penetration efficiency. On removal of the patch, none of the tips were broken as observed under the microscope.

Furthermore, Simvastatin microneedles successfully penetrated to a depth of around 200 µm, while TH microneedles penetrated to about 80 µm demonstrating their ability to deliver their respective payload within the epidermis and upper dermis (**Figure 6(d-e)**). This placement is likely similar to what would be expected in human skin, considering the comparable thickness of both tissues.

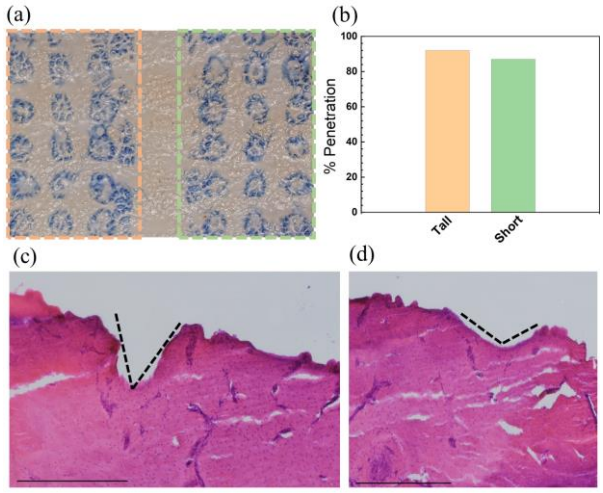

Figure 6: Microneedle penetration studies in pig skin model and its analysis (a) Trypan blue staining of microneedle insertion wounds in pig skin (Scale bar = 0.5mm) The wound marks within the orange rectangle are made by the tall microneedles (Simvastatin loaded), while the ones within the green rectangle are made by the shorter microneedles (TH loaded). (b) Penetration efficiency of the tall and the short microneedles shown separately on the pig skin (d) H&E-stained section of pig skin penetrated by the microneedle array. The insertion wound is indicated by the black dashed line (Scale bar = 0.5mm) (c) Tall microneedles (d) Short microneedles.

CONCLUSION

In conclusion, we present a scalable approach to fabricate a large array of microneedles of different dimensions on the same patch. This was achieved by precisely adjusting laser parameters such as speed, power, and focus. Changing the dimensions resulted in unique drug release profiles for each drug. In our demonstration, we utilize this to tailor independent release of two drugs: simvastatin and tetracycline hydrochloride simultaneously each with their unique release profile. In general platform can be scaled to delivery more than 2 drugs and achieve arbitrary release profiles. Moreover, this platform enables targeted delivery of drugs to different depths within the skin. The arrays can also be made as large as small as needed and can be customized to the shape of underlying tissue.

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