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Fatih Inci *et al.*

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A wearable 3D-printed hollow microneedle device for pressure-driven interstitial fluid collection and testing

 Nedim Hacıosmanoğlu, ^{ab} Emre Ece ^{ab} and Fatih Inci ^{*ab}

Interstitial fluid (ISF) is the extracellular fluid within the dermis that transports biomolecules diffusing from blood vessels to lymphatic vessels. Owing to its blood-like composition and accessibility only a few millimeters beneath the skin surface, ISF has recently attracted considerable attention as a minimally invasive reservoir for biomarker analysis. Conventional ISF collection relies on invasive sampling methods. Microneedle (MN) technology has emerged as a promising approach for developing minimally invasive ISF sampling and analysis systems. We present a design and one-step stereolithography (SLA)-based 3D printing fabrication of a wearable hollow MN device: μ HolloSense. The device is capable of negative pressure-assisted ISF collection via its syringe port and is compatible with lateral flow assay (LFA) testing through a dedicated test port. The overall cost was $\sim$ \\$1 per single-use device, including all components, and \\$1.50 per SARS-CoV-2 antigen test, used here as a proof-of-concept LFA system. Additionally, a 3D-printed, agarose-based skin-mimicking platform was developed to provide a standardized tool for evaluating MN sampling performance. Beyond this model system, *ex vivo* skin experiments were conducted to validate the applicability of μ HolloSense for ISF collection in biologically relevant tissues. Our results demonstrate that μ HolloSense, featuring a refined tip diameter ($44.19 \pm 2.4 \mu\text{m}$) and height ($1207.93 \pm 11.25 \mu\text{m}$), is capable of drawing liquids at a rate sufficient to reach the dermis, exhibiting robust mechanical properties ($>0.17 \text{ N}$ compressive force per needle) in IgG LFA tests across antigen concentrations of 1, 10, 100, and 250 pg mL^{-1} . *Ex vivo* experiments on mice skin confirmed ISF extraction of up to $6 \mu\text{L}$ per sampling, with protein concentrations consistent with physiological levels. Collectively, this work presents a unified strategy for the design, fabrication, and evaluation of 3D-printed hollow MN systems with an integrated negative-pressure approach for ISF-based biomolecule analysis. In the future, further optimization and clinical validation of this platform would enable continuous, minimally invasive monitoring of a wide range of biomarkers, paving the way for point-of-care diagnostic and personalized health applications.

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1. Introduction

Body fluids are the crucial reservoirs of metabolic information that reflect the chemical and physiological state of the human body.^{1–6} In clinical practice, blood plasma has become the gold standard for diagnostic, therapeutic, and monitoring applications due to its rich biomolecular content.^{7–12} Although there is significant knowledge on blood analysis and many available products, blood collection requires trained medical personnel and expensive device facilities for in-depth analysis.^{13,14} Even if rapid diagnostic tests are applied, blood sampling poses risks of infection and is not

sustainable for continuous monitoring applications due to invasive sampling conditions and widespread needle phobia in the population.¹⁵

Recently, interstitial fluid (ISF) has gained attention as a promising medium for biomarker detection, particularly in wearable diagnostic platforms. Its collection is relatively simpler than blood because the skin provides easy access and contains an ISF reservoir distributed across different layers.^{16–18} ISF is mainly presented in the dermis layer of the skin and is composed of diffused biomolecules between blood vessels and lymph vessels via blood plasma, which originates from capillaries and surrounds the cells to transfer metabolites between cells and cellular waste to lymph capillaries.¹⁹ As a result of continuous molecular exchange with vasculature, ISF contains a spectrum of blood-derived analytes such as glucose, proteins, antibodies, and metabolites, positioning it as a robust matrix for

^a UNAM-National Nanotechnology Research Center, Bilkent University, 06800, Ankara, Turkey. E-mail: finci@bilkent.edu.tr

^b Institute of Materials Science and Nanotechnology, Bilkent University, 06800, Ankara, Turkey

physiological monitoring. Yet, ISF does not exhibit clotting, which creates an advantage for microfluidic systems and continuous monitoring applications.^{20–24} In omics studies, it has also been demonstrated that plasma and ISF contain 79% common and 1% ISF-specific biomarkers.²⁵ Although the concentration of metabolites, especially antigens, is not as high in serum as in the ISF, diagnostic tests can be developed for various applications.²⁶ The dermis layer of the skin is only 1 mm beneath the skin surface, below the stratum corneum and epidermis, so retrieval and diagnostic procedures for ISF can also be achieved with minimally invasive methodologies.^{27,28} Due to such special positioning, ISF has an immense diagnostic value for reflecting the plasma content. Different techniques have been developed to collect ISF with non-invasive methodologies, including suction blisters and reverse iontophoresis. On the other hand, sampling with microneedles (MNs) has gained attention due to its effectiveness and simplicity during applications.¹⁶ The COVID-19 pandemic also revealed the significance of MN-based testing strategies due to simplicity of the methodology and pain-free nature of sampling. Various studies have been conducted on SARS-CoV-2 or other viral antigens or antibody retrieval from serum and ISF, which contains proteins similar to those in blood.^{22,25,28–34}

MNs are basically structures ranging from 25 to 2000 μm in height that can penetrate the stratum corneum and reach the epidermis layer of the skin without damaging blood vessels or stimulating nerve endings. Due to this minimally invasive nature, MNs are widely utilized in transdermal drug delivery and biosensing applications.^{35,36} Being available in many different shapes, aspect ratios, and material types, MNs (especially hollow MNs) provide an advanced strategy for ISF collection without invasive approaches and the possibility of self-operation for users.^{37–46} Despite their promising features, design and large-scale production of MNs remain critical challenges for widespread biomedical applications.^{47,48} In addition, requirement of expensive production facilities for conventional MN production strategies such as lithography and chemical etching also increases application costs and prevents accessibility of MN technology for users.⁴⁹ Advances in additive manufacturing and 3D printing technologies provide a promising path for producing MNs and other microstructures in large scale, allowing complex designs to incorporate functional elements such as electronics and pumps to the system.^{50–52} This budget-friendly technique also promises a wide range of polymers for production.^{53,54} Considering all of these factors, developing a wearable ISF collection and testing system with 3D-printed MNs has potential to overcome the limitations of standard technologies for physiological monitoring.

In this work, we designed and produced a wearable, easy-to-use, minimally invasive ISF extraction device: $\mu\text{HolloSense}$. We characterized physical and proof-of-concept device properties. In order to achieve this, we designed a 3D model of our prototype device and printed out the device using an SLA-based resin printer. Subsequently, the device dimensions

were verified using various characterization techniques, including scanning electron microscopy (SEM), fluorescence light microscopy, and topography mapping (KEYENCE VK-X100). Mechanical properties were evaluated using dynamic mechanical analysis (DMA). After that, a standardizable agarose-based ISF-like skin model was developed to evaluate the efficiency of the system using the SARS-CoV-2 nucleocapsid protein as a model antigen for an in-device lateral flow assay (LFA). Finally, $\mu\text{HolloSense}$ was tested on *ex vivo* mice skin to characterize the mechanism of skin penetration, ISF extraction performance and physiological relevance. As a result, a wearable ISF-extraction device prototype was developed, offering a novel solution to the aforementioned challenges.

2. Materials and methods

2.1. 3D Design and pre-printing

All 3D structures were designed using Shapr3D software. The finalized constructs were exported as .stl files for slicing before 3D printing. These files were then imported into CHITUBOX software, which was adjusted to the pre-installed Halot ONE (Crealty, China) resin printer specifications. A layer height of 50 μm (Z-axis resolution) was used for all prints, with an exposure time of 3.4 s and a bottom layer exposure time of 65 s. All remaining parameters were maintained at the default settings. Sliced structures were exported as .cxdlp files from CHITUBOX software, which were then transferred to the 3D printer using a generic USB flash drive.

2.2. 3D printing and post-processing

Printing designed parts were made with plant-based ultraviolet (UV) resin (Anycubic, SKU: AC-R10080, China) or class-I medical-grade resin (Alias, SKU: 09DS610009, Türkiye), both of which were polymerized under a 405 nm wavelength light source. Anycubic resin is primarily composed of 2,2'-(ethylenedioxy)diethyl diacrylate (monomer and crosslinker), triethylene glycol diacrylate (increaser of crosslink density), glycerol propoxylated esters with acrylic acid (help tailor resin viscosity), and phenyl bis(2,4,6-trimethylbenzoyl)-phosphine oxide (photoinitiator). The initiator system used in this resin included the photoinitiator phenyl bis(2,4,6-trimethylbenzoyl)-phosphine oxide, which activates under UV light (405 nm). While 405 nm is at the boundary between the UV-A band (315–400 nm) and visible violet light, these resins are named as “UV-curable resins” in industrial and academic literature.^{55–57} Detailed information on chemical composition is not publicly available for Alias resin, but it is a class-I medical-grade material certified under ISO 13485:2016 and ISO 9001:2015 quality standards (<https://tr.alias3dresin.com/products/alias-3d-printing-dental-surgical-guide-resin/>). After completion of the printing cycle, structures were cleaned with 100% of isopropanol (IPA), sonicated for 5–15 min in IPA, and subsequently rinsed with IPA to remove uncured resin. To ensure the internal channels of the hollow MNs remained open and free of obstruction, a syringe filled with IPA was

used to apply pressure through the MN bores, while all other openings were sealed during the process. We considered that any residual resin was polymerized during post-curing stage, and that the residual IPA on the structure evaporated with the heating of the same stage.

2.3. SEM

The surface morphology and hollow tip structure of MNs were characterized using an environmental scanning electron microscope (Quanta 200 F, FEI, USA). Prior to imaging, samples were coated with a 10 nm-thick conductive layer of gold–palladium (Au/Pd) using a Gatan Model 682 Precision Etching and Coating System (Allest Instruments, USA). This coating minimized surface charging and improved image clarity under the electron beam. MN patches were mounted on aluminum stubs using conductive carbon tape and positioned at a 30° tilt angle to facilitate visualization of the needles, needle tips, and bore openings. SEM was performed under high-vacuum conditions at an accelerating voltage of 10 kV and a spot size of 3.0, enabling high-resolution assessment of external geometry and hollow outlet features.

2.4. Keyence analysis

The preliminary dimensional characterization of the fabricated hollow MNs was performed using a 3D laser scanning microscope (VK-X100, Keyence, Japan). This system enables high-resolution, layer-by-layer scanning along the Z-axis at the nanometer scale, providing accurate topographical and dimensional data. For the analysis, an MN patch was positioned under the microscope objective, and measurements were conducted to determine key geometrical parameters such as the height, base width, tip diameter, and inner hollow diameter of the MN. A 10× objective lens was used to measure the height, base width, and tip diameter of the MNs. SEM was performed with a Z-axis resolution of 1 μm after selecting appropriate upper and lower bounds for each needle. Then, the acquired data were processed using VK-Analyzer software, where dimensional parameters were extracted. Measurements were conducted on five individual needles across a single patch ($n = 5$) to evaluate dimensional uniformity and reproducibility. The bore diameter was measured separately using a 20× objective lens to improve visualization of the internal cavity. Similar to the previous analysis, a Z-axis resolution of 1 μm was used. The bore width was quantified using VK-Analyzer and subsequently compared with the theoretical CAD design values to assess dimensional fidelity.

2.5. Light microscopy-fluorescence imaging

An EVOS M5000 microscope (Thermo Fisher, USA) was used to image needle penetration and fluorescence. In order to minimize variability, all images were taken at 1.25× magnification and 0.100 light adjustment.

2.6. Mold and skin model preparation

A skin-mimicking agarose test platform was also 3D-printed following the instructions given in sections 2.1. and 2.2. To produce an agarose gel with ISF-like properties, we prepared 2.65% (w/v) agarose with 5% (v/v) glycerol, 10% (v/v) artificial human serum (Thermo Fisher, USA), 1% (w/v) bovine serum albumin (BSA) and SARS-CoV-2 nucleocapsid protein (N protein) as a model antigen target.

2.7. DMA

The mechanical performance of hollow MNs was evaluated using a dynamic mechanical analyzer (TA Instruments, Q800, USA) in compression mode. Two distinct test setups were employed to assess the structural integrity under axial load and penetration capability into a tissue-mimicking substrate. In the first setup, individual MN patches were placed directly on the compression platform. A compressive force of 18 N was applied at a constant loading rate of 0.5 N min^{−1} to evaluate the static mechanical resistance of MNs. Displacement–force data were recorded to assess deformation behavior and failure thresholds. In the second setup, to simulate insertion into soft tissue, the MN was attached to the upper movable clamp using adhesive, while 2.6% (w/v) agarose gel was placed on the lower fixed platform to serve as a skin purpose. The MN was driven into the agarose under the same compression protocol. In addition to displacement–static force data, relaxation modulus values were recorded to evaluate the time-dependent mechanical behavior of the system and quantify penetration efficiency under viscoelastic conditions. The resulting data were analyzed to compare the compression strength and insertion performance of the hollow MN. In the last experimental set, *ex vivo* mice skin was used to test skin penetration force by placement at the lower fixed platform instead of the agarose model. The resulting displacement–force data were collected for 500 μm advancement into the skin.

2.8. Assembling μHolloSense

In order to assemble the device, a medical-grade adhesive band (3M) was cut to the specified dimensions (Fig. S3C) using a laser cutter (LazerFix, Türkiye) with 30% power and 50% speed settings. After that, the adhesive band was placed with the adhesive side facing down towards the MN side and affixed to the 3D-printed component using 3M double-sided adhesive tape. Lastly, a sterile 5 mL syringe was inserted into the syringe port, which fitted tightly to complete the device assembly. Cost/component analysis for bench-scale production of a single μHolloSense and stopper (excluding overhead costs) is given in Table S1.

2.9. SARS-CoV-2 antigen test

The N protein antigen was tested using a skin-like agarose gel containing an N protein concentration of 1, 10, 100, or 250 pg mL^{−1}. Before experimentation, the agarose mold was

cleaned with IPA, and the skin-like agarose mixture (1.5% agarose, 5% human serum albumin (ALBA®, USA), 5% glycerol, and varying concentrations of recombinant SARS-CoV-2 N protein (Pro-Sci, USA) inside 1× phosphate-buffered saline (PBS)) was poured into the mold. After solidification, the agarose slab was removed and soaked in PBS-ISF buffer (1× PBS with 5% human serum albumin, 5% glycerol, and varying concentrations of recombinant SARS-CoV-2 N protein) for 2 h to provide hydration. Before sampling, the soaked gel was gently blotted. The assembled μ HolloSense was inserted into the agarose, a vacuum was applied using a syringe, and suction continued for 5 min. After that, the extracted liquid was diluted with antigen test reaction buffer (1 mL), and an LFA strip (NOVACHECK (Türkiye) SARS-CoV-2 Antigen Rapid Self-Test) was inserted into the μ HolloSense reservoir. For each antigen concentration, three replicates of antigen tests were to compare the results. Images of LFA strips were captured using a smartphone camera (iPhone 13 Pro, Apple, USA), and band intensities were quantified using ImageJ software, following previously reported protocols.⁵⁸

2.10. Ethics and *ex vivo* animal experiments

Ex vivo animal experiments were conducted under the approved ethical protocol (protocol no. 2025/20) granted by Bilkent University Animal Experiments Ethics Committee (BILHAYDEK). Visualization of the MN insertion site was performed using trypan blue staining on mice skin. Nude mice (eight weeks old, female) were terminated by exposure to diethyl ether for 3–4 min, followed by cervical dislocation. The dorsal skin (3 × 5 cm) was excised with a scalpel and then immersed in PBS with 1% penicillin–streptomycin until use. The ISF extraction experiment was conducted by first wiping skin samples from PBS and mounting them on a 5 cm diameter, 2 mm-thick polymethyl methacrylate (PMMA) support. After that, μ HolloSense was applied to the skin under thumb pressure for 1 min, ensuring complete adhesion of the medical tape. Then, to provide negative pressure, the syringe was drawn to the 5 mL mark, and a stopper was placed to stabilize the pressure for an additional 5 min. To measure volume and protein concentration, ISF samples were flushed onto the skin by applying positive pressure with the syringe after extraction. ISF samples were then collected from the surface with a micropipette and measured by discarding air until the tip was filled with ISF to a certain level. Characterization of ISF protein concentration was performed using a Qubit (Thermo Fisher, USA) fluorometer with the Protein Broad Range (BR) Assay Kit. Before visualization for the MN section, punctured skin samples were immersed in trypan blue solution for 30 s. Before sectioning, skin tissues were fixed in 4% paraformaldehyde (in 1× PBS, pH 7.4) at 4 °C for 18 h, then washed in 1× PBS to be embedded in 3% agarose with the epidermal side facing upward. After that, agarose blocks were briefly chilled on ice to solidify and then sectioned at a thickness of 200 μ m using a vibratome device (Leica VT 1200

S, Germany). Tissue sections were collected during the procedure and visualized under a 200× electronic microscope (Andonstar, China).

3. Results and discussion

3.1. Device design and production

Current ISF sampling techniques face limitations that slow the transfer of ISF-based diagnostics and real-time analysis to the clinic.⁵⁹ Depending on the technique, primary methodologies such as suction blistering, microdialysis, reverse iontophoresis, and solid MNs with suction often suffer from low sample volume, susceptibility to contamination with sweat and other skin remnants, and low reproducibility of isolation efficiency.⁶⁰ In addition, suction-based methodologies require additional tools or machinery to provide consistent pressure.²⁵ These limitations prevent ISF from being used as a standardized target for the accurate quantification of biomarkers. A hollow MN platform with a low-cost, easy-to-integrate, and built-in suction system could solve the abovementioned limitations. Furthermore, a single-use, sterile device with an active sampling strategy could yield new methodologies for ISF testing, including real-time monitoring and on-site quantification.

The overall design of the μ HolloSense incorporates a combination of features that provide multiple functions in a single unit (Fig. 1A), thereby addressing the limitations of current technologies. MN design incorporated in our device (Fig. 1B) aimed to have 2.5:1 (height:width) aspect ratio (Fig. S1A), consisting of 88 pyramidal-shaped MNs (Fig. S1D) each designed as 1650 μ m in height, 650 μ m in width, and containing a bore with a diameter of 360 μ m spanning from the tip to the base (Fig. S1B). The MN geometry consists of a square pyramidal structure (base: 650 × 650 μ m²; height: 1650 μ m) with a 360 μ m cylindrical bore. This configuration results in a variable sidewall thickness ranging from 150 μ m at the needle base to 0 μ m at the bore exit height of 75 μ m. Each MN was separated by 1 mm distance from their square-shaped base (Fig. S1C) and placed on top of a reservoir. This reservoir collects the ISF extracted through the hollow MN bores and directs it to the buffer/LFA port (Fig. 1D) and syringe port (Fig. 1E). These two ports have specific dimensions for insertion to provide suction for ISF retrieval, dripping buffer, and inserting an LFA strip to operate antigen tests (Fig. S1E and F). The final 3D-printed μ HolloSense successfully reproduced the designed features from the design, which were also confirmed with physical characterizations (Fig. 1C and F).

3.2. Device characterization (electron microscopy and SEM)

Initial characterization of the μ HolloSense device was operated with a 200× electronic microscope (Andonstar, China) to visualize and confirm printed geometry of the MNs (Fig. 2A), hollow MN bores (Fig. 2B), and the formation of open inlets to these 2.25 mm-long internal holes, which

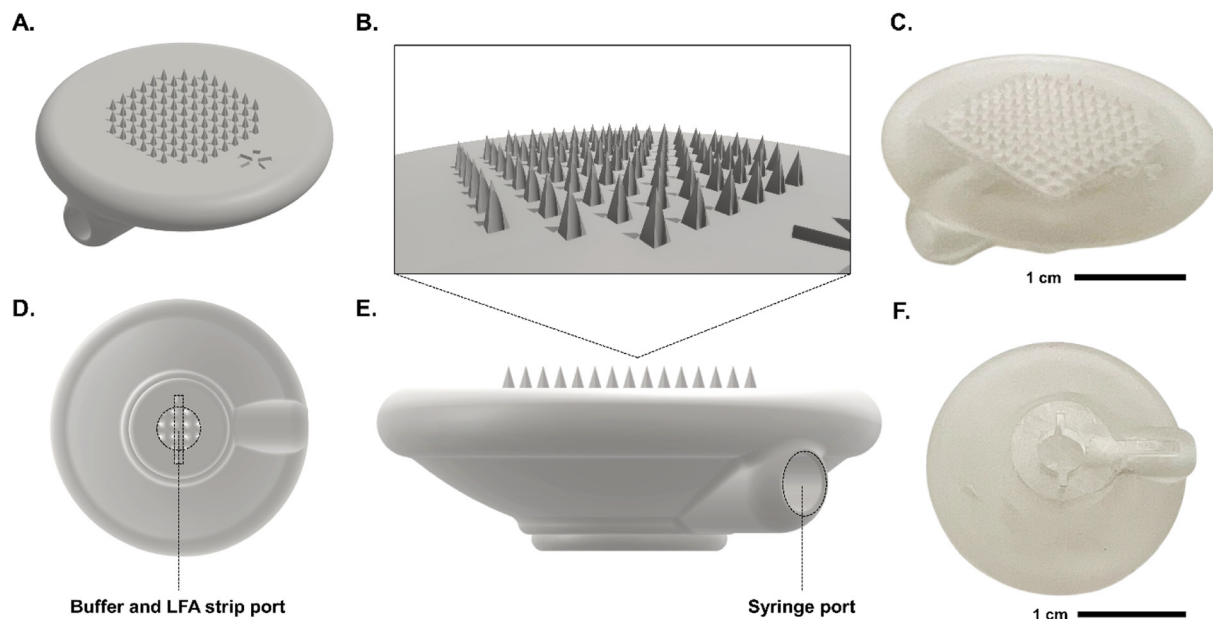


Fig. 1 Design and fabrication of μ HolloSense. (A) Overall design. (B) MN shape and orientation. (C) 3D-printed μ HolloSense (front view). (D) Buffer and LFA port. (E) Syringe port. (F) 3D-printed μ HolloSense (back view).

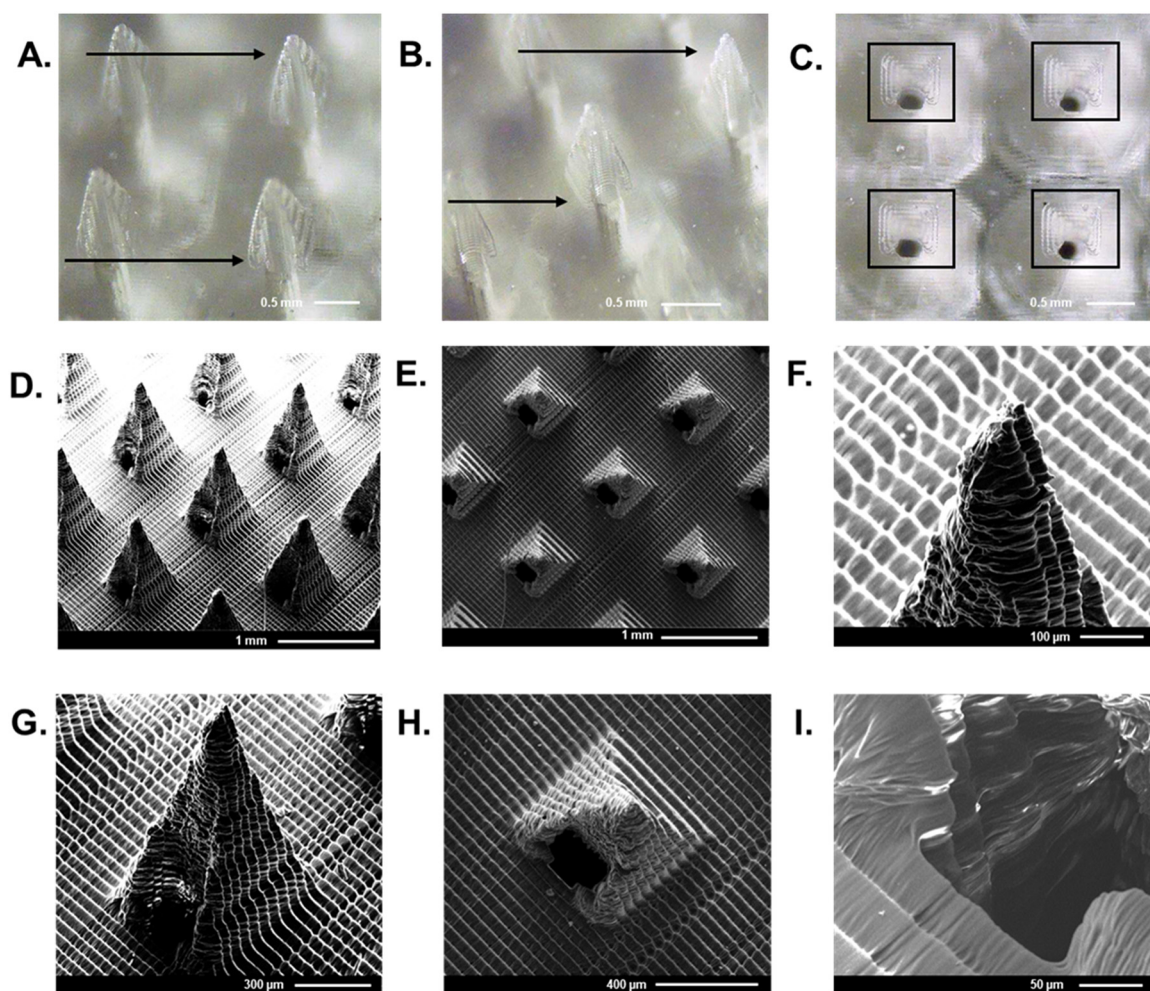


Fig. 2 Characterization of μ HolloSense performed with an optical microscope (A–C) and scanning electron microscope (D–I). (A) 3D-printed MNs. (B) Microfluidic channels. (C) MN bores. (D) Multiple MNs – side. (E) Multiple MNs – top. (F) MN tip. (G) MN bore – side view. (H) MN bore – top view. (I) MN bore – close up. Arrows and squares show the focus of interest.

connect the surface of the μ HolloSense to its reservoir (Fig. 2C). After inspection of these features, SEM images of device features were also visualized. We confirmed that the 3D orientation of the device features was obtained as expected (Fig. 2D). However, the entrance of the hollow MN bores exhibited slight elliptical distortion due to printer resolution limitations (Fig. 2E). Nevertheless, the tip diameter of printed MNs was $<50\text{ }\mu\text{m}$ (Fig. 2F) and positioned to not prevent MN tip formation (Fig. 2G and H). SEM also revealed that, although printing layers became visible as features became smaller (Fig. 2I), the overall structure maintained its functional integrity. The hollow MN bores remained open, and the tip sharpness was preserved in the final construct, indicating that the resolution of the 3D printer did not critically affect device performance.

3.3. Device characterization (Keyence VK-X100)

A more quantitative measurement of the 3D-printed features of the μ HolloSense was conducted using the Laser Confocal Non-Contact Profilometer (Keyence VK-X100, Japan). The height of individual MNs (Fig. 3A), the entrance dimensions of the hollow MN bores (Fig. 3B and C), and tip diameters were quantified from five randomly selected MNs. Fig. 3D revealed that five heights were measured ($1192.55\text{ }\mu\text{m}$, $1203.96\text{ }\mu\text{m}$, $1207.43\text{ }\mu\text{m}$, $1208.31\text{ }\mu\text{m}$, and $1227.44\text{ }\mu\text{m}$), resulting in an average height of $1207.93 \pm 11.25\text{ }\mu\text{m}$. The

corresponding base widths were $683.7\text{ }\mu\text{m}$, $680.76\text{ }\mu\text{m}$, $674.85\text{ }\mu\text{m}$, $670.42\text{ }\mu\text{m}$, and $698.48\text{ }\mu\text{m}$, yielding an average base width of $681.64 \pm 9.59\text{ }\mu\text{m}$. Tip diameters were measured as $47.85\text{ }\mu\text{m}$, $43.82\text{ }\mu\text{m}$, $40.39\text{ }\mu\text{m}$, $43.46\text{ }\mu\text{m}$, and $45.43\text{ }\mu\text{m}$, resulting in an average tip diameter of $44.19 \pm 2.40\text{ }\mu\text{m}$. According to these results, the relative printing tolerances were calculated to be 0.93% for MN height (Z-axis), 1.41% for base width (X-Y plane), and 5.43% for tip diameter. The comparatively higher variability observed in tip diameter was attributed to the combined influence of lateral (X-Y) resolution and vertical (Z-axis) layer discretization during 3D printing. Compared with the original design parameters, a dimensional deviation of 4.87% was observed for base width, whereas a substantially higher deviation of 26.79% was identified for MN height. This pronounced height deviation was primarily associated with limitations in the Z-axis resolution of the 3D printer, including layer-by-layer fabrication effects and minor mechanical inaccuracies. Overall, the μ HolloSense MNs achieved an aspect ratio of $\sim 2:1$. Prior to the design stage, the literature reports employing 3D-printed MN structures were comprehensively analyzed to determine the optimal geometric parameters for the μ HolloSense platform. Based on this analysis, a height range of $1000\text{--}1500\text{ }\mu\text{m}$ was identified as sufficient for effective ISF extraction, even assuming a penetration depth of only 50–60%.^{16,30,41,61} Furthermore, studies have commonly reported MN widths of $250\text{--}650\text{ }\mu\text{m}$. Taking into account the

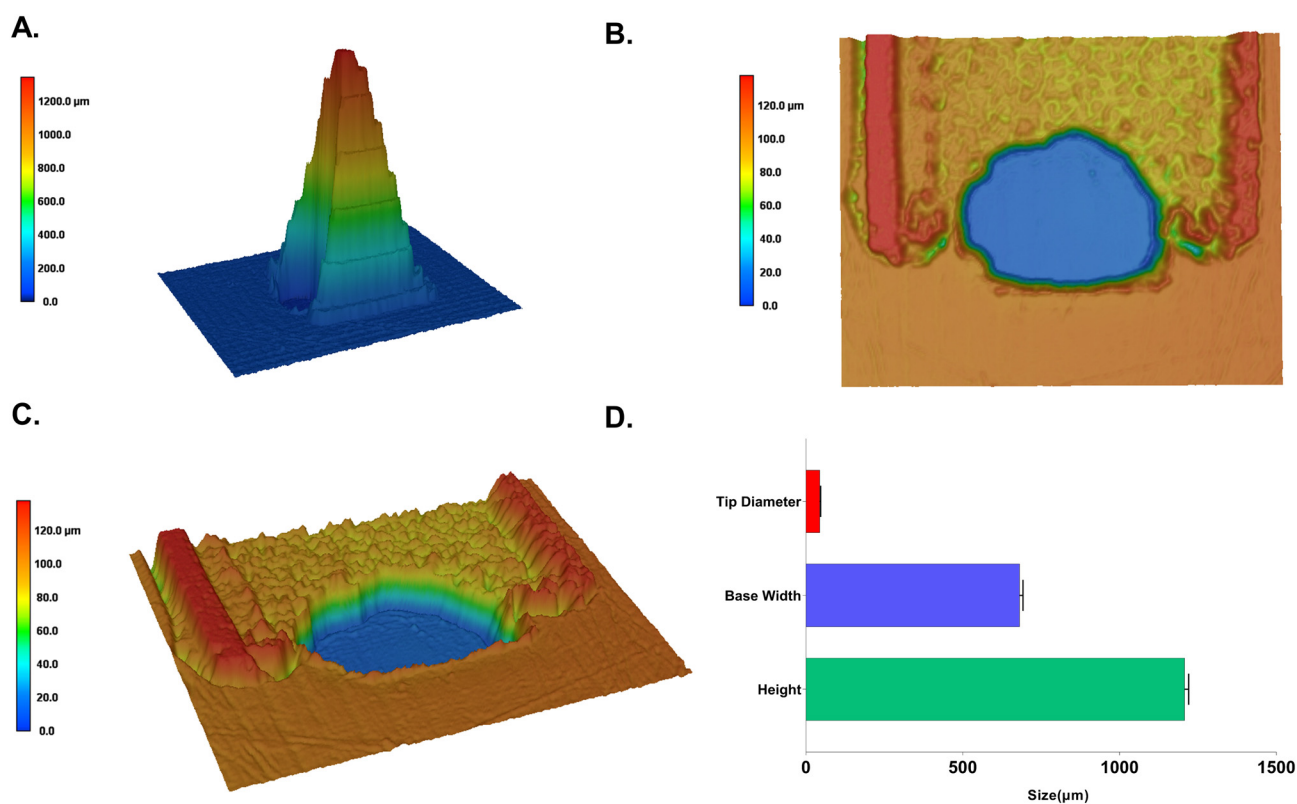


Fig. 3 Laser confocal non-contact profilometer characterization of μ HolloSense. (A) Tip size. (B) Hollow MN bore entrance size from top and (C) side and (D) measured feature sizes.

20–30% shrinkage observed along the Z-axis (height direction) during 3D printing and post-curing processes (Fig. 3), the final μ HolloSense design was defined as a pyramidal MN array with an edge length of 650 μm and nominal height of 1600 μm .

3.4. Device characterization (DMA)

Mechanical analysis of μ HolloSense was performed using two distinct modes of measurement. First, elastic compression of bare MNs was measured by DMA (Fig. 4Ai and ii). We concluded that a static force of >6 N was required to break μ HolloSense MNs, which was higher than the penetration force required to pierce skin and higher than that reported for most polymeric MNs (Fig. 4B). Then, the skin piercing force of the MNs was measured using an agarose gel slab which represents the mechanical strength of the skin, as previously characterized. A 2×2 cm agarose slab (2.65% w/v) was prepared, and DMA was performed to determine the piercing force and relaxation modulus. A static force <1 N was sufficient to pierce the skin-mimicking agarose (Fig. 4C), and the relaxation modulus at the piercing point was measured to be <0.02 MPa (Fig. 4D). Overall, the results confirmed that our model mimicked the skin to a certain degree, and that the force required to pierce the skin was relatively low compared with that in the literature.⁶²

3.5. Skin-mimicking agarose model production and testing

Testing wearable devices requires standardized test platforms which also provide support elements for medical tapes that

are used to stabilize these devices and could support suction tests. In order to overcome these limitations, a skin-mimicking agarose test mold was designed (Fig. 5A) and produced (Fig. 5B) with dimensions matching the μ HolloSense MN orientation (Fig. S2).

3.6. Skin-mimicking agarose ISF model LFA test

A model antigen test for μ HolloSense was designed to operate with a pre-built skin-mimicking agarose ISF model (Fig. 6A). In order to achieve a realistic model, previously characterized, a physiologically relevant serum antigen concentration was selected for ISF, which was 30–50% diluted compared with serum and contained serum proteins.⁶³ After preparation of 1, 10, 100, and 250 pg mL^{-1} agarose models, LFA tests for SARS-CoV-2 N protein antigen were performed with fully assembled μ HolloSense (Fig. S3). Testing procedures began by taping the assembled μ HolloSense device (Fig. S3A and B) onto the pre-cast agarose test platform (Fig. S4A) using a laser-cut adhesive bandage (Fig. S3C), which was affixed with 3M double-sided adhesive tape. A syringe equipped with a 3D-printed stopper (Fig. S3D and E) was then inserted into the designated port to apply suction. After a 20 min negative-pressure application period for liquid extraction, 1 mL of assay buffer from LFA test kit was dripped from the buffer port (Fig. S4B) and the LFA strip was also inserted from the same opening (Fig. S4C), which was sealed with tape during suction. LFA test results showed concentration-dependent band formations (Fig. 6B), which were measured

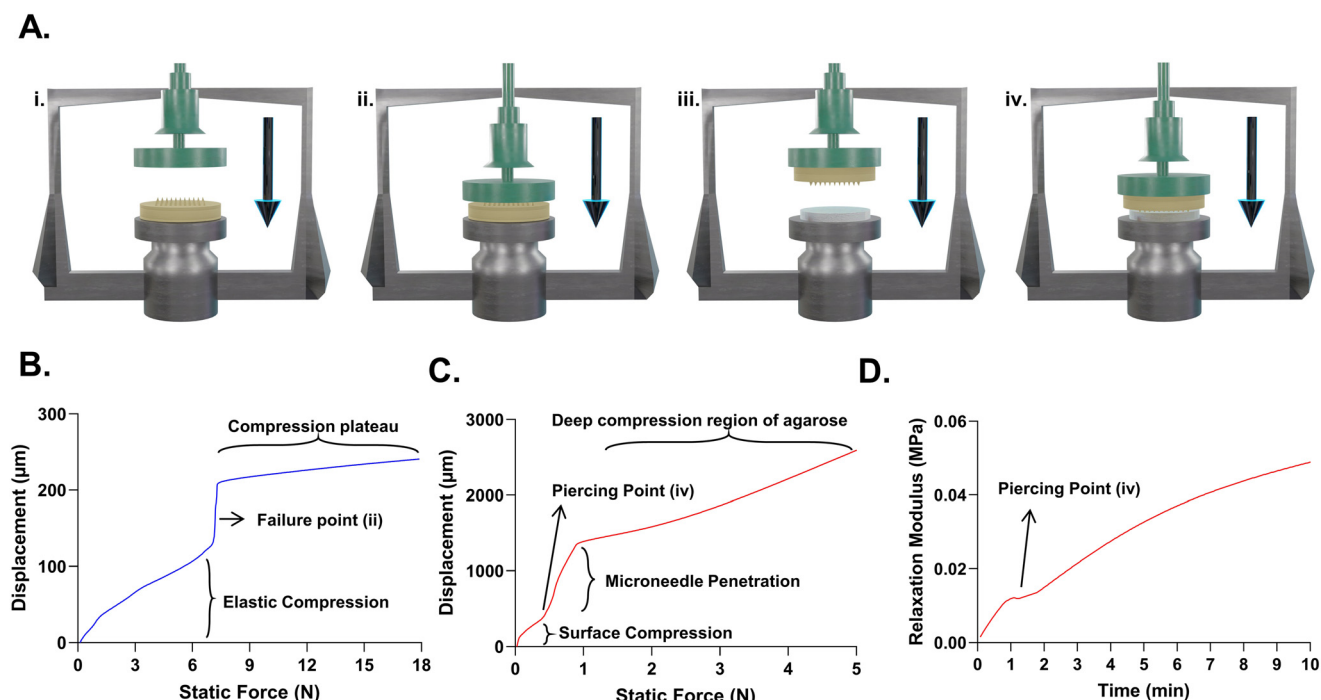


Fig. 4 DMA for μ HolloSense. (A) Modes of measurement (Ai) and (Aii) needle analysis. (Aiii) and (Aiv) agarose piercing analysis. (B) Needle compression. (C) MN penetration of agarose model. (D) Relaxation modulus.

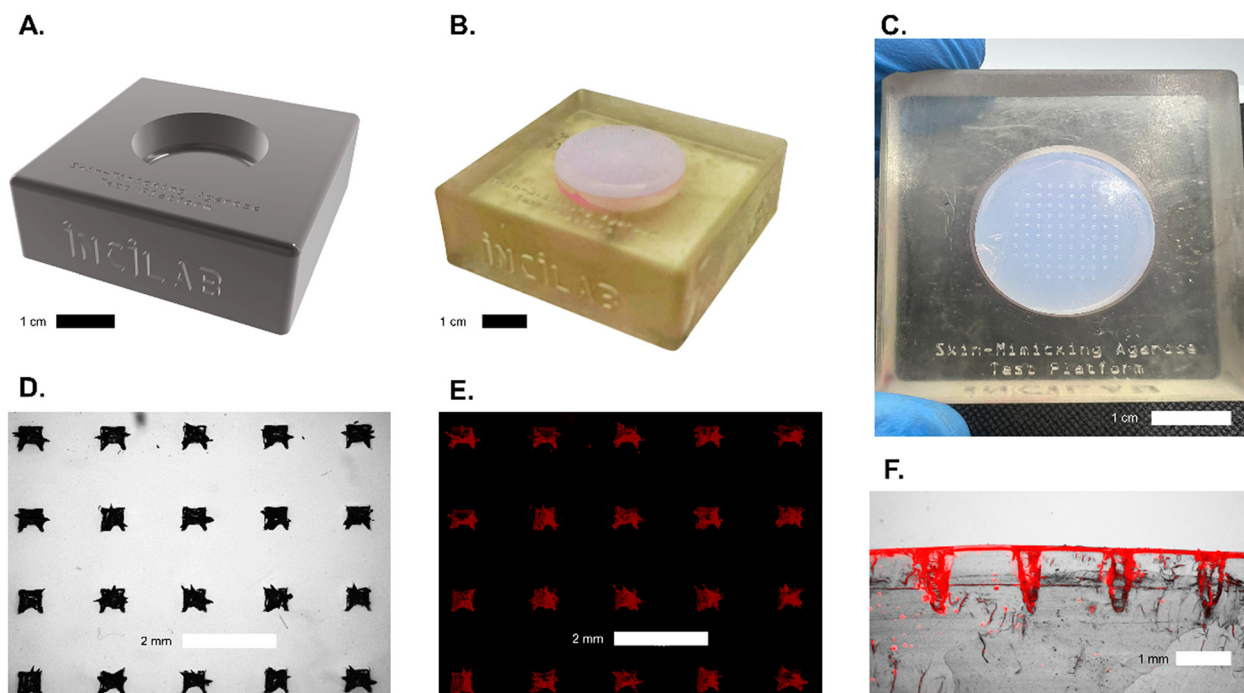


Fig. 5 Agarose skin model and penetration test for μ HolloSense. (A) 3D mold design. (B) 3D-printed mold and skin-mimicking agarose. (C) MN-penetrated agarose. (D) MN penetration – light microscope. (E) MN penetration – fluorescence microscope. (F) MN penetration – merged light and fluorescent image.

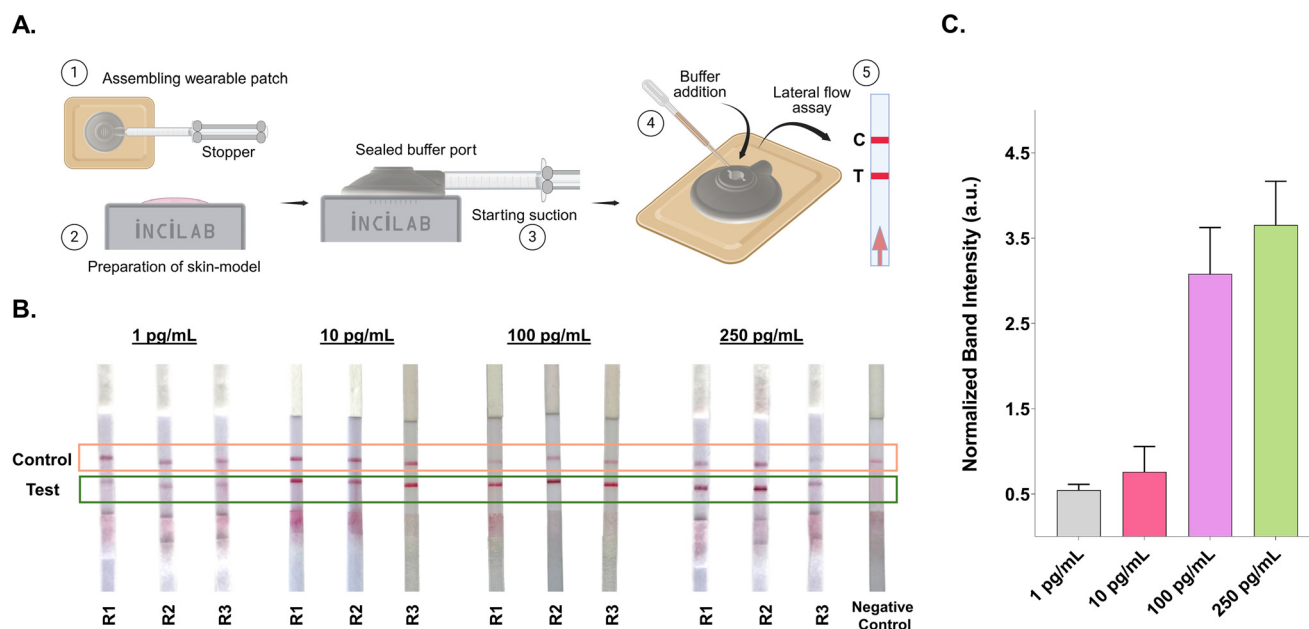


Fig. 6 μ HolloSense protocol and model antigen test. (A) Five-step experimental workflow. (B) LFA test results. (C) ImageJ band intensity analysis result for LFA bands.

with NIH ImageJ band intensity analysis for the SARS-CoV-2 N-protein analyte (Fig. 6C). As a result, μ HolloSense retrieved fluid from the skin mimicking model with more than 2-fold signal change for a 10-fold concentration increase. A purified and pre-verified antigen diluted in an

inorganic buffer was used for the tests with a commercial LFA platform. Hence, false positives (FP) or false negatives (FN) were not observed during the test. Therefore, an estimation of the accuracy would not yield a realistic measurement to show the true diagnostic accuracy of the

μ HolloSense in clinical settings or real-world applications. SARS-CoV-2 N protein antigen was selected arbitrarily to demonstrate a model case for antigen detection through ISF sampling and LFA assay, considering the wide availability of N protein and SARS-CoV-2 antigen tests. The literature shows that viral or bacterial antigens can be transferred to the ISF after infection, as a result of the ISF being a physiological representation of blood plasma.^{17,19} Our main aim was to demonstrate a cost-effective and easy-to-obtain viral antigen detection setup for future experimental effort which could become a standard test strategy in possible future pandemics. The concentration of pathogen-specific antigens in ISF is governed by multiple factors, including antigen molecular weight and capillary permeability which, together, regulate molecular exchange between the vasculature and interstitial compartment.⁶⁴ Protein antigens derived from viral and bacterial pathogens typically partition from the bloodstream into ISF at approximately 10–25% of their plasma concentrations, consistent with the overall protein composition of ISF relative to plasma.^{26,28,65} Emerging evidence indicates that this partitioning ratio is dynamic and can vary with viral load and disease progression, with antigen concentrations frequently observed in the picogram per milliliter (pg mL^{-1}) range. Recent studies have demonstrated that SARS-CoV-2 antigens are detectable in plasma and ISF at pg mL^{-1} levels, underscoring the feasibility of leveraging ISF as an alternative biofluid for antigen detection.^{63,66,67}

In the present study, a panel of SARS-CoV-2 N protein antigens at physiologically relevant pg mL^{-1} concentrations was selected for evaluation using a commercially available LFA kit, reflecting the clinical importance of N protein detection and the widespread accessibility of antigen-based diagnostics. Beyond immediate applicability, this strategy offers a cost-effective and experimentally tractable framework for the development of ISF-based model antigen detection systems, thereby supporting future efforts to advance minimally invasive diagnostic technologies. With over 500 proteins and some ISF-specific proteins, ISF is a rich source of biomarkers that could be used as targets for monitoring various diseases.⁶⁸ A broader inspection of the ISF could also be adopted for various disease conditions, such as diabetes mellitus, cancer, and other chronic diseases, to identify additional biomarkers that can be transferred from capillaries to the skin ISF.^{69–71}

3.7. *Ex vivo* animal experiments

Lastly, *ex vivo* animal experiments were performed with skin samples obtained from nude mice. After obtaining ethics committee approval, skin samples were harvested from the animals, which had already been used for a different experiment unrelated to ISF content or volume. With this strategy, no excess animals were sacrificed for our study, which also aligns with the replacement, reduction, refinement (3R) principle.⁷² To ensure the biocompatibility of

our device, we reproduced it with the same dimensions using a class-I, biocompatible resin (Fig. 7A and B), which could also be autoclaved before use. This resin also meets ISO 10993-5:2009 (non-cytotoxic polymer), ISO 10993-10:2010/(R)2014 (non-irritating and non-sensitizing polymer), and complies with EN ISO 13485:2016 and EN ISO 14971:2012 biocompatibility certificates. The mechanical strength required for skin penetration was evaluated using a DMA setup. The skin sample was fixed to the lower stage and the μ HolloSense device positioned on the movable upper head which was operated with a force of 18 N. A force of <6 N was sufficient to achieve a 500 μm penetration depth, which is well below the 40 N force which is typically exerted by thumb pressure (Fig. 7C).⁷³ Complete MN insertion was confirmed by the visible imprint pattern on the skin surface (Fig. 7D) and cross-sectional histology, which verified stratum corneum penetration, a prerequisite for ISF extraction (Fig. 7E). In order to evaluate the ISF extraction volume and measure the protein concentration from extracted samples, excised mice skin was placed on top of a solid PMMA support (Fig. 8A). ISF extraction was initiated with insertion of the MN array into the skin by thumb pressure, and completely sealing the medical tape on the skin to prevent air leakage. A control test was also performed with pierced skin without negative pressure application to show that ISF extraction was strongly dependent upon the suction pressure applied (Fig. 8B). Application of negative pressure on the pierced skin was achieved by drawing the syringe up to the 5 mL mark, and placing the stopper to retain the pressure. After 5 min, in order to measure the exact volume of extracted ISF, air and liquid trapped within the device was flushed onto the skin and collected with a micropipette (Fig. 8C). Replicated experiments showed that 5.77 ± 0.33 μL of ISF could be extracted with a single μ HolloSense sampling (Fig. 8D), which also yielded a concentration of 21.04 ± 0.82 $\mu\text{g mL}^{-1}$ (Fig. 8E).

Although the μ HolloSense platform presents an advanced, integrated approach for ISF extraction, it remains at the proof-of-concept stage, and certain limitations persist that warrant future optimization. First, μ HolloSense has so far been demonstrated only for single-time sampling. To enable continuous sampling, MN placement and ISF filling dynamics will require further refinement. Second, as a prototype device, μ HolloSense has undergone only proof-of-concept characterization. Hence, future *in vivo* studies in animal and human models may necessitate additional structural modifications, such as resizing the MN array support or incorporating a peripheral pressure ring. In addition, large-scale production of the device may require high-throughput production strategies such as injection molding or micro-molding. Finally, the ISF collection volume of μ HolloSense was estimated to be approximately 6–7 μL , which is comparable to values reported for MN-based extraction systems.^{74,75} The limited volume observed in our study was primarily attributed to ISF dilution during skin preservation in PBS and the lack of blood circulation to renew ISF in the *ex vivo* model.¹⁷

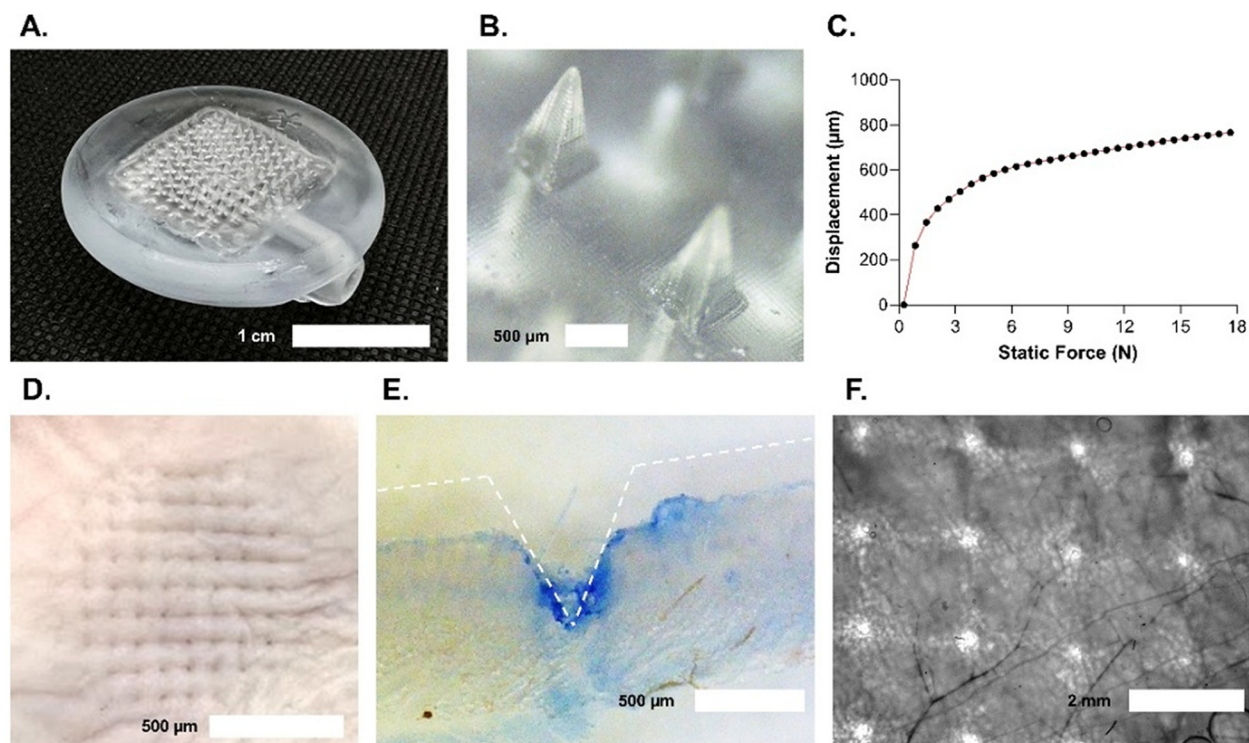


Fig. 7 Production of μ HolloSense with medical-grade resin and mouse skin tests. (A) μ HolloSense device made with class-I resin. (B) MN and bore structures. (C) Mouse skin penetration using DMA. (D) Mouse skin after DMA. (E) Profile of mouse skin penetration. (F) Mouse skin penetration pattern under a light microscope.

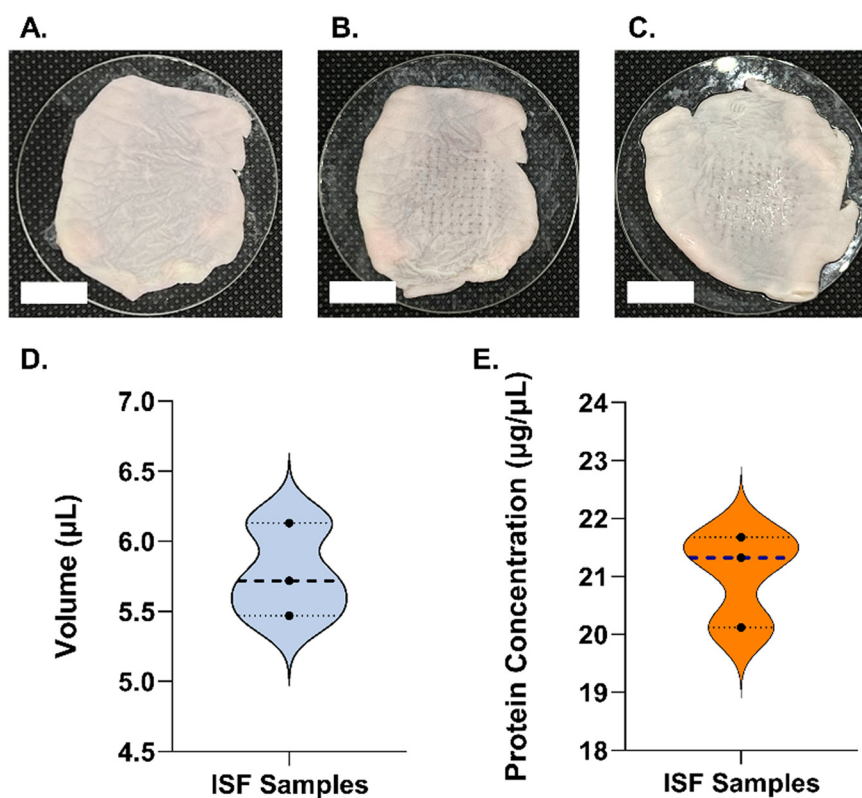


Fig. 8 Mouse skin ISF extraction tests. (A) Before penetration. (B) Penetration without suction. (C) Penetration with suction. (D) Extracted ISF volume. (E) Extracted ISF protein concentration. Scale bar = 1 cm.

4. Conclusions

A low-cost, easy-to-operate, and non-invasive ISF sampling prototype device, μ HolloSense, was designed, 3D printed, characterized, and carried out for a biomedical application. Although vacuum-assisted ISF sampling using MNs is not a new concept, our work introduces a fully integrated, low-cost, and easily operable device that is entirely 3D-printable. This platform enables rapid prototyping of future ISF-based diagnostic assays and offers a distinctive cost-to-performance advantage relative to previously reported systems (Table S2). Validation experiments conducted using a SARS-CoV-2 nucleocapsid antigen spiked agarose model demonstrated the effective integration of μ HolloSense into a skin-mimicking antigen testing platform. Additionally, all auxiliary components, including a custom-designed syringe stopper, were also 3D-printed, offering a standardized and modular approach for future MN-based sampling systems. Finally, μ HolloSense was produced from medical-grade resin and tested with *ex vivo* mice skin to show ISF extraction volume and concentration per sample as well as mechanical analysis for skin penetration. Recent studies have reported passive sampling devices with ISF extraction capabilities, typically based on multi-component architectures and diffusion-driven, vacuum-free passive sampling mechanisms.^{76–78} In contrast, the device presented here adopts a single-step, fully integrated design that can be fabricated using a low-cost 3D printer, while enabling tunable control over sampling duration and extracted ISF volume. In future studies, μ HolloSense would be adapted for *in vivo* animal and human testing following design modifications. To enhance ISF extraction efficiency, a compression ring would be incorporated around the MN array to apply localized pressure to surrounding tissue and medical tapes with better adhesive will also be adopted to the system. 3D printing strategies with high-resolution polymerization techniques, including two photon polymerization (2PP) and liquid interface production, could also be used to produce such systems with improved surface roughness and microstructure positions.^{43,79} Besides, a sampling strategy developed with μ HolloSense could be adopted to detect target proteins with organ-on-a-chip systems for advanced biosensing applications.^{80,81} On the other hand, microfluidic and plasmonic sensor systems could also be incorporated with μ HolloSense for downstream detection of proteins and other pathogenic markers.^{82–85} These advances would support the translation of μ HolloSense into practical biomedical applications and contribute to the broader standardization of MN-based diagnostics.

Author contributions

Fatih Inci conceptualized the study. Nedim Haciosmanoğlu designed the device, skin test mold, and conducted the 3D printing experiments for further analyses. Emre Ece performed SEM, Keyence, and all DMA experiments. Nedim Haciosmanoğlu designed all the auxiliaries of the platform,

performed fluorescence and light microscopy experiments, assembled the device, performed LFA tests and *ex vivo* animal experiments. Fatih Inci secured funding and led the project. All authors contributed to finalization of the manuscript.

Conflicts of interest

Authors declare no conflict of interest.

Data availability

Data are available upon request.

Supplementary information (SI): Supplementary information file that includes design and dimensions for the μ HolloSense, skin-mimicking agarose test platform and the accessory parts, device assembly and operation, cost/component analysis and literature comparison. See DOI: <https://doi.org/10.1039/d5lc00657k>.

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