

Implementation of Sterilization and Disinfection Protocols for Potentiometric Microneedle Sensors: A Requirement for a Safety Assertion

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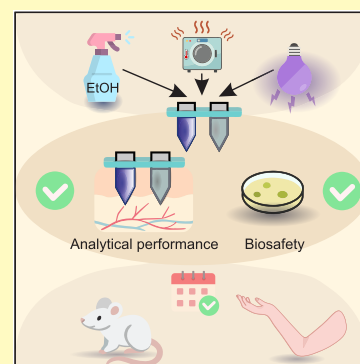
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Supporting Information

ABSTRACT: Microneedle (MN) sensors represent a promising technology for disease surveillance. For their application in long-term animal studies and preclinical research, they must exhibit both analytical reliability and biosafety, including the elimination of microorganisms capable of inducing infection. Nonetheless, MNs' compatibility with conventional sterilization and disinfection methods remains largely unexplored. Accordingly, we introduce the first systematic evaluation of the compatibility of potentiometric MN sensors with sterilization and high-level disinfection protocols widely available in laboratory settings, including ethanol, autoclave, and UV-C exposure. As a proof-of-concept, we assess the impact of these three protocols on the analytical performance of a complete potentiometric cell in the MN configuration (i.e., pH-MN based on tridodecylamine as hydrogen ionophore and polyurethane as polymer matrix and polyvinyl butyral-Ag/AgCl reference electrode MN). Ethanol treatment preserved the calibration parameters of the sensor, maintaining a near-Nernstian response even after prolonged exposure (30 min), with minimal change in the slope (58.9 vs 57.9 mV pH⁻¹). Slight variations were observed in the standard potential (E^0), although all differences remained below 5%. In contrast, autoclaving severely compromised both MN electrodes, confirming incompatibility with our pH-MN design. Then, UV-C was further optimized through a two-step exposure protocol, resulting in the maintenance of the sensor accuracy with <4% deviation from a reference pH value. After confirming analytical reliability, microbiological assays were conducted to evaluate the microbial inactivation effectiveness of ethanol and UV-C protocols. Both treatments successfully eliminated bacterial contamination even in sensors with a high level of contamination, supporting their suitability for preclinical use. Finally, both protocols were validated for intradermal pH sensing in *ex vivo* skin, showing excellent agreement (<4% deviation) with a commercial pH electrode. Overall, we present a systematic validation workflow to assess disinfection and sterilization method compatibility with polymer-membrane ion-selective microneedle patches. The workflow can provide a basis for designing analogous tests for other microneedle-based potentiometric sensors and potentially also in other sensing principles.

KEYWORDS: potentiometric sensors, microneedles, sterilization, disinfection, pH sensing



The increasing demand for continuous and minimally invasive health monitoring is driving the development of next-generation sensing technologies. Microneedle (MN)-based sensors represent a transformative platform for the real-time monitoring of clinically relevant analytes, paving the way for personalized medicine and remote patient care. Although their potential is incalculable, most MN sensors remain yet at an early stage of development in terms of human validation, limited by critical bottlenecks such as ethical approval, regulatory certification, and the lack of standardized validation protocols.¹

A typical roadmap to assess MN-based sensors toward human research shall consist of long-term *in vivo* evaluation, initially with long-term experiments in animal models and subsequently in human subjects.² MNs are devices that breach the skin barrier; thereby presenting several biological challenges such as cytotoxicity, immune activation, fibrotic encapsulation, and increased risk of infection due to potential pathogen

infiltration into underlying tissues.^{3–5} These factors can compromise both device performance and patient safety. Therefore, achieving effective disinfection and sterilization without compromising device functionality is critical in the development of MN-based technologies. International regulatory standards, such as ISO 10993 and ISO 11139, emphasize user safety by requiring both biocompatibility and validated sterilization or disinfection procedures. While biocompatibility testing (particularly cytotoxicity) is commonly assessed in the early

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development, disinfection protocols are often overlooked in emerging technologies like MN sensors, and typically addressed only in later stages, during regulatory approval.⁶

According to the ISO 11139:2018 regulation, sterilization is a process used to render a product free from viable microorganisms, while disinfection refers to a process intended to kill or remove microorganisms, excluding bacterial spores.^{7,8} Procedures commonly used to eliminate microbial contamination—such as gamma irradiation, autoclaving, ethylene oxide gas, vaporized hydrogen peroxide, or ultraviolet-C light (UV-C, radiation covering wavelengths between 100 nm to 280 nm)—can significantly alter the properties of the device, ultimately compromising its performance. For example, Galante et al. reported alterations in sterilized hydrogels designed for biomedical applications,⁹ and Plaxco et al. noted functional deterioration in planar aptamer-based sensors following certain disinfection procedures.¹⁰ Noteworthy that these challenges are not exclusive to emerging technologies: even in well-established platforms such as glucose strips compatible with commercial glucometers, the difficulty of preserving sensor functionality after sterilization has persisted for decades, and several methods such as ethylene oxide and gamma irradiation have been explored.^{11,12} Moreover, the transition to continuous glucose monitoring (CGM) systems has introduced new challenges, including breaching the skin barrier for extended periods, which further increases the demands for biocompatibility, stability, and infection control. These aspects also play a critical role in the context of microneedle (MN)-based devices, which similarly rely on penetrating the skin to access interstitial fluid or deliver therapeutics.

Notwithstanding these challenges and the increasing necessity to evaluate MN-based devices in preclinical and exploratory clinical contexts,^{13–18} high-level disinfection and broader safety assessments remain inconsistently addressed in early-stage MN research. Common practices such as ethanol or UV disinfection are often used without assessing their impact on sensor performance, and microbial inactivation are frequently unvalidated or substituted without clear justification.¹⁹ Only a limited number of studies examine potential alterations in the sensor functioning after the proper treatment, and formal disinfection or sterility assurance according to pharmacopeial standards typically lacks.^{20,21} This critical gap underscores the need to rigorously address sterilization in the development of MN-based sensors, evaluating not only safety but also the impact of these procedures on device integrity and performance.

Potentiometric MN sensor is a very interesting system to investigate the impact of sterilization or disinfection on MN-based sensors' performance and integrity. First, potentiometric sensors target clinically important analytes in interstitial fluid (ISF), such as electrolytes and gases, positioning them as valuable tools for continuous health monitoring.^{22,23} In addition, they feature a simple device architecture and lack biological recognition elements, which reduces complexity and makes them more suitable for early-stage validation studies, thereby making them a strategic starting point for evaluating sterilization compatibility in MN-based sensing platforms.

Herein, we characterize for the first time the compatibility of potentiometric MN sensors with sterilization and high-level disinfection techniques, which is expected to serve as a foundation for future protocols applicable to other chemical MN sensor configurations. Thus, we primarily focused on commonly available and easily implemented techniques in standard laboratory settings, such as autoclaving, chemical disinfection with ethanol,

and physical methods like UV-C exposure. Recent reports indicate that certain planar potentiometric electrode designs, such as solid-contact pH-sensitive electrodes based on covalently crosslinked polymeric membranes, are compatible with autoclave sterilization and can withstand stress conditions such as elevated temperatures or exposure to organic solvents.^{24,25} Other methods, such as gamma irradiation, while potentially suitable for sterilizing planar potentiometric sensors (e.g., plasticized PVC-based pH-selective electrodes and thin-film Ag/AgCl reference electrodes), are not considered in this study due to their restricted availability and the practical limitations they pose for routine laboratory implementation.^{26,27} We selected pH-MN sensors previously developed and tested by our group, due to their clinical relevance and robustness.²⁸ These sensors have been successfully applied in both *ex vivo* and *in vivo* contexts, demonstrating accurate pH determination in interstitial fluid and mechanical resilience to repeated skin insertions, making them a relevant and representative platform for evaluating sterilization options.

■ EXPERIMENTAL SECTION

MN-Sensor Fabrication

The pH-MN patches used in this work were prepared as previously described by our group.²⁸ Briefly, the pH-MN patch consisted of two solid stainless steel MNs embedded into a parafilm support. These MNs were then modified to function as the working electrode (WE-MN) and reference electrode (RE-MN), respectively. The WE-MN follows a layered structure, starting with applying a conductive carbon ink layer onto the needle. This was followed by the deposition of functionalized multiwalled carbon nanotubes (f-MWCNTs), which serve as an ion-to-electron transducer.^{29,30} Finally, a plasticized polyurethane hydrogen-selective membrane (HSM) based on tridodecylamine as hydrogen ionophore was applied in three layers to ensure complete and uniform coverage, with drying steps between each application. On the other hand, the RE was created by coating a Ag/AgCl-modified needle with a reference membrane made from poly(vinyl butyral) (PVB) saturated with NaCl, which ensures stable chloride concentration at the solid-state interface.³¹ After overnight conditioning the RE-MN in a highly concentrated NaCl solution (3 M), a polyurethane (PU) overlayer is applied to enhance the membrane's stability. The fabrication process is outlined in Figure S1, with further details available in the Supporting Information. Representative images of the completed pH-MN patch, as well as the WE and RE, are shown in Figure S2. Prior to utilization, the electrodes were preconditioned overnight in aqueous solutions: 1 mM HCl for the MN-WE and 3 M NaCl for the MN-RE.

Analytical Parameters

Electrochemical assessment of the pH-MN sensors before and after sterilization was performed using a custom setup in which the MN patches were connected to a high-impedance input ($10^{15} \Omega$) 16-channel potentiometer (EMF16, Lawson Labs, Inc.) via BNC cables and electrical clamps. Potentiometric measurements were conducted at room temperature ($23 \pm 1^\circ \text{C}$) with continuous agitation set at 300 rpm using a magnetic stirrer (IKA COLOR SQUID S000, IKA, Germany).

Sterilization and Disinfection Procedures

We applied sterilization and disinfection procedures to the MN sensors as follows. First, the sensors were fabricated and electrochemically interrogated to ensure proper functionality. They were then subjected to a certain sterilization or high-level disinfection treatment. Finally, the electrochemical performance was immediately re-evaluated to assess the impact of each procedure, without any additional post-treatment. The

specific treatments along with the corresponding parameters, are summarized in Table 1.

Evaluation of Treatment Efficiency

To prove the microbiological effectiveness of the treatments, we performed two complementary assessments. In the first approach, after treatment, sensors were placed onto sterile Luria-Bertani (LB) agar plates and were incubated overnight at 37 °C. The presence of visible bacterial growth around the sensors was recorded as a positive result, indicating that the sterilization or disinfection procedure failed to eliminate viable microorganisms. Absence of growth was considered a negative result, confirming the effectiveness of the treatment. In the second approach, a more stringent assay was utilized. The MN sensors were deliberately contaminated with *E. coli* and subsequently subjected to either sterilization or disinfection treatments. The treated sensors were then transferred to fresh sterile media and incubated under controlled conditions to allow potential bacterial regrowth. Serial dilutions of the incubation medium were placed onto agar plates and monitored for colony-forming units (CFUs) counting after overnight incubation. The Supporting Information provides full experimental details for both procedures.

Ex Vivo Assays

To assess the suitability of the treated MN patches for the detection of intradermal pH levels, *ex vivo* measurements were conducted on rat skin samples preconditioned at specified pH values. The pH was assessed by piercing the skin samples with the MN patch and utilizing then a calibration (with a protocol properly established for each procedure) to calculate the pH from the potential recording. Measurements were validated by comparison with intradermal readings accomplished with an ultra-micro pH meter. Additional experimental details are provided in the Supporting Information. Rat skin samples were donated by the University Miguel Hernández (Alicante) from animals used in other unrelated studies. No animals were sacrificed specifically for the purposes of this research.

RESULTS AND DISCUSSION

We developed a multi-step experimental methodology to examine the compatibility of potentiometric MN sensors with various sterilization and disinfection methods. The study incorporates the evaluation of the analytical performance of the sensor, sterility validation, and assessment of the accuracy for intradermal measurements based on rat skin samples. Figure 1 depicts the workflow developed to understand any possible influence of each sterilization treatment on the analytical performance, encompassing sensitivity, reversibility and response time. Concurrently, we confirmed that the treated sensors addressed defined sterility standards as well as evaluated their biocompatibility. Finally, the MN sensors were utilized for *ex vivo* measurements in rat skin samples, hence targeting their appropriateness for future biological applications.

Effect of Sterilization and Disinfection Treatments on the Analytical Performance of Potentiometric MN-Based Sensors

Utilization of Ethanol. We investigated the compatibility of the pH-MN sensor with sterilization based on a disinfection strategy using ethanol, which is a common antiseptic. Ethanol works by denaturing proteins, disrupting cell membranes, and inactivating viruses and bacteria. Its effectiveness depends on both concentration and exposure time.³² Typically, 70% ethanol is optimal for microbial inactivation, with exposure times of 1–2 min being sufficient for most bacteria and viruses.^{32,33} However, higher disinfection levels, particularly for spores and resistant viruses, may require 10–30 min, depending on the surface nature.³³ Note that ethanol is most effective on non-porous surfaces, and its efficiency decreases on porous or polymeric materials, such as those used in MN sensors.³³ Then, in the

Table 1. Sterilizing and Disinfecting Techniques and the Tested Conditions

treatment	type	conditions	duration	temperature	considerations
ethanol	disinfection	70% (v/v)	10 or 30 min	RT ^b	air-dried before testing
steam sterilization (autoclave)	sterilization	121 °C, 15 psi	20 min	121 °C	one cycle
UV irradiation – single cycle	high-level disinfection/sterilization	$\lambda = 254$ nm	10 or 30 min	RT ^b	two-sided exposure ^a
UV irradiation – double cycle	high-level disinfection/sterilization	$\lambda = 254$ nm	(a) total time: 10 min (2 cycles $\times$ 5 min each); (b) total time: 30 min (2 cycles $\times$ 5 min each).	RT ^b	two-sided exposure per cycle ^a

^aTwo-sided UV exposure was accomplished by manually inverting the sensor to irradiate both its upper and lower surfaces, ensuring consistent biocidal coverage. ^bRT: room temperature, 25 $\pm$ 1 °C.

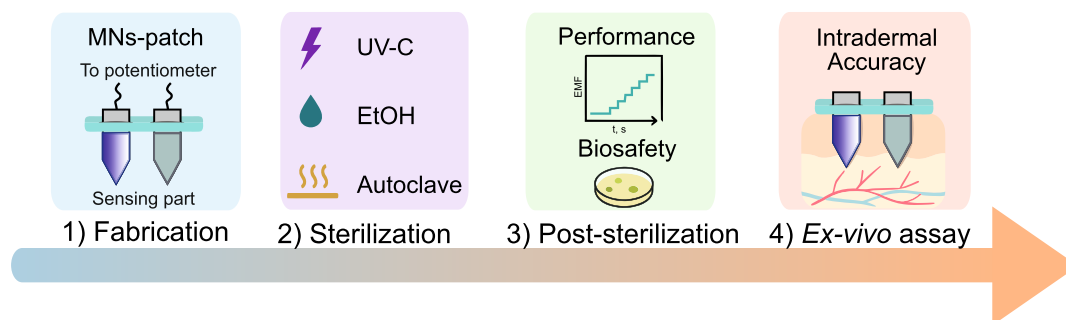


Figure 1. Illustration of the experimental methodology employed in this study to evaluate the compatibility of various disinfection and sterilizing protocols with potentiometric MN-based sensors.

particular case of plasticized polymeric membranes as those herein used, ethanol may alter its integrity, e.g., by extracting/dissolving any of the components, especially at high concentrations and long exposure times.³⁴

We exposed the MN sensors to 70% ethanol for 10 and 30 min, both exposure times being expected to provide high disinfection levels. While the 10-min-treatment resembles standard protocols, the 30 min one involves a more aggressive procedure that might be required for specific sensor designs or high-risk contamination scenarios. Also, this approach allowed us to assess whether prolonged exposure compromises either the analytical performance or structural integrity of the sensors compared to shorter, routine methods.

First, we focused on evaluating the WE-MN response. The sensor was calibrated against a commercial double-junction Ag/AgCl reference electrode in a series of buffer solutions with different pH values (from 5.0 to 8.5 buffer solutions). Thereafter, the sensor was exposed to ethanol and recalibrated once a stable potential was reached (i.e., <5 min and ~1 h after 10 min and 30 min ethanol exposure, respectively). Figure S3 shows a comparison of two calibration curves obtained before and after the ethanol treatment. As observed, the sensor maintained its near-Nernstian response for both 10- and 30-min exposure times. For the 10-min treatment, the average slope ($n = 3$) was 56.2 mV dec^{-1} before treatment and 55.6 mV dec^{-1} after it, representing a slight difference (Change %) of 1.1%. Also, for the 30-min treatment ($n = 3$), the slopes revealed no substantial deviation (58.9 vs 57.9 mV dec^{-1}). Minor variations were observed in the standard potential (E^0) for both exposure times (Change % < 5%), although the difference was slightly higher at a longer exposure. Statistical analysis using a paired sample t-test indicated no significant differences in either the slope ($p = 0.8$ for 10 min ($n = 9$), $p = 0.75$ for 30 min ($n = 9$)) or E^0 ($p = 0.7$ for 10 min ($n = 9$), $p = 0.72$ for 30 min ($n = 9$)), with a threshold of $p < 0.05$. Furthermore, sensors subjected to both treatments exhibited comparable linear ranges of responses (LRRs) (pH 5.0 to 8.5), covering the physiological pH window, as well as similar, fast response times ($t_{95} < 10 \text{ s}$).

Subsequently, we incorporated the RE-MN together with the WE-MN, resulting in the complete MN patch (see Figure S1), and we run the ethanol disinfection protocol for 10 min and 30 min. Figures 2a and Figure 2b show representative calibration curves from a patch before and after ethanol treatment. Calibrations were repeated three times before and after treatment using the same patch to evaluate repeatability and potential deviations in slope and intercept under significantly drastic conditions. No notable differences were observed between the consecutive cycles: the curves were found to maintain a near-Nernstian behavior, with Changes % in the slope and intercept of <5% before and after treatment. Similar results were observed when various patches were analyzed ($n = 11$). Figure S4 and Table S1 summarize the slope and intercept values of all the patches before and after ethanol exposure. Most of them showed a minimal variation in the calibration slope and intercept, with a change % < 5%. Only two patches exhibited slightly higher changes (i.e., >5%), particularly for the intercept values. Considering the results in the experiment based on the untreated external reference, it can be concluded that the WE response remained stable, and any observed shift is most likely attributable to the RE rather than the WE. Overall, these effects were negligible, indicating that ethanol disinfection (70% v/v, 10–30 min) does not compromise sensor stability or repeatability. Additionally, we checked the reversibility of the

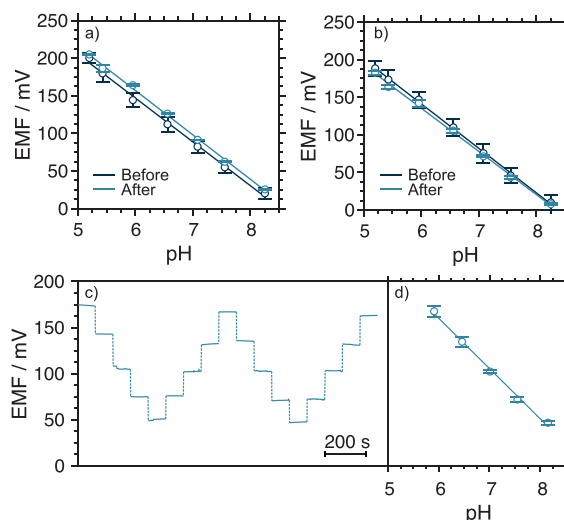


Figure 2. Evaluation of the effect of ethanol treatment on pH-MN sensor performance. (a) Average calibration graph obtained with the pH-MN sensor before and after exposure to ethanol treatment for 10 min ($n = 3$). (b) Average calibration graph obtained with the pH-MN sensor before and after exposure to ethanol treatment for 30 min ($n = 3$). (c) Reversibility study of the pH-MN signal after 30 min of ethanol treatment. The pH of the sample was sequentially changed in the following order: 6.0 → 6.5 → 7.0 → 7.5 → 8.0 and then 8.0 → 7.5 → 7.0 → 6.5 → 6.0. (d) Averaged calibration graph.

patch facing the process based on 30 min of ethanol treatment by recording two successive calibration curves with alternating decreasing and increasing pH values (Figure 3c). The sensor exhibited slightly greater variation compared to the untreated condition, with relative standard deviations (RSD%) of 3.2% for the slope and 4.1% for the intercept. Nevertheless, this level of

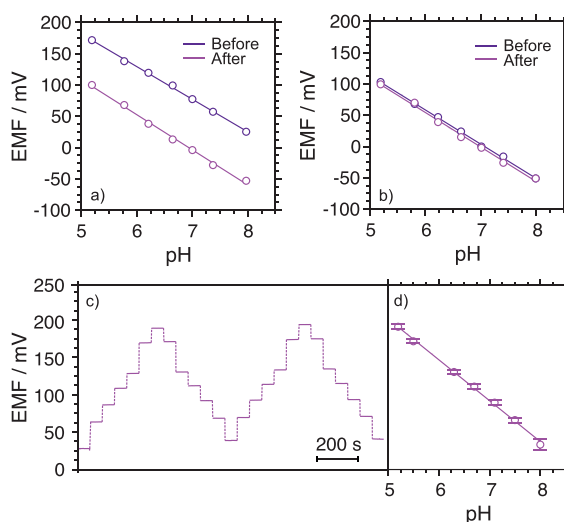


Figure 3. Evaluation of the effect of repeated UV-C irradiation on pH-MN sensor performance. (a) Calibration graphs obtained with the same pH-MN sensor before and after a first UV-C irradiation cycle of 15 min. (b) Calibration graphs obtained with the same pH-MN sensor used in (a) before and after a second UV-C irradiation cycle of 15 min. (c) Reversibility study of the pH-MN signal after the second UV-C cycle. The pH of the sample was sequentially changed in the following order: 5.0 → 5.5 → 6.0 → 6.5 → 7.0 → 7.5 → 8.0 → 7.5 → 7.0 → 6.5 → 6.0 → 5.5 → 5.0. (d) Corresponding averaged calibration curve.

reversibility remains adequate to enable *in vivo* measurements across a broad pH range. Moreover, a better reversibility is expected using a shorter ethanol exposure time. These results indicate that the functional integrity of the microneedle sensor is preserved after ethanol treatment. Optical microscopy images confirmed that the surface morphology and structural integrity remained unaltered, with no visible modifications in either the WE-MN or RE-MN after ethanol treatment (Figure S5).

Utilization of Steam Sterilization (Autoclave). We explored another widely used sterilization method: steam sterilization or autoclaving. Notably, the process provides easy availability, cost-effectiveness, and proven ability to eliminate a broad range of microorganisms.³⁵ However, it is here anticipated that, the high temperatures, pressures and prolonged exposure associated with this procedure was found to significantly compromised the potentiometric response of the MN sensors. As illustrated in Figure S6a (see Table 1 for details about the used experimental conditions), autoclaving three WE-MN sensors resulted in a notable worsening of the performance, with marked changes in both sensitivity and standard potential of the corresponding calibrations. Moreover, the deterioration followed a random, non-systematic pattern, with no consistent trend across sensors. On average, the WE-MN showed a marked and highly variable deviation in slope, decreasing from -51.6 ± 3.4 mV/dec ($n = 3$) to a range from -52.4 to -8.3 mV/dec. The RE-MN was integrated with a WE-MN, and the assembled patch was sterilized. In all cases ($n = 3$), no measurable EMF signal was obtained after autoclave treatment. Optical micrographs (Figure S6b) revealed severe structural damage to the RE-MN, including extensive melting of the membrane. However, the WE-MN showed a less pronounced tip blunting and surface erosion. The lesser degree of structural damage may explain why the WE-MN retains measurable function in some cases. So, the autoclaving approach in its current form, following typical laboratory sterilization conditions (121 °C, 20 min), is not suitable for the practical application of this sensor and was therefore not adopted for further experiments in the present work.

However, even if autoclaving cannot be employed in the present case, this limitation is specific to the materials and device architecture employed here. Alternative polymer matrices (e.g., plasticizer-free membranes), degrees of plasticization, or non-polymeric ISEs architectures may offer higher resistance to steam-induced damage, and should be evaluated individually for each sensor design.

In addition, alternative steam-sterilization strategies could also be explored, including shorter high-temperature cycles (e.g., 3–5 min at 134 °C) or sterilization of the sensor while immersed in a liquid medium (e.g., PBS), as potential approaches to mitigate thermal and mechanical stress.

Utilization of UV-C Light. Evaluating UV-C treatment for wearable sensors and sensitive medical devices is highly relevant, as it offers a low-temperature, chemical-free alternative to traditional sterilization methods.³⁶ UV-C is effective against bacteria and viruses and it typically does not degrade materials.^{12,32,36,37} While UV-C sterilization is easy to access and implement,³⁶ its antimicrobial efficacy in practical applications depends on factors such as dosage, wavelength, geometry, and material transparency, and it is inherently limited to surface-based actuation.

First, we tested the influence of UV-C exposure on the WE-MN potentiometric response, following a similar approach to that used for ethanol disinfection. Two different durations

(10 and 30 min) were selected. Importantly, exposure times shorter than 10 min have been reported as sufficient for the inactivation of most bacterial species, while longer durations, such as 30 min, are commonly employed for highly contaminated surfaces or greater source-to-target distances.^{38–40} Figure S7 shows representative calibration curves of two individual WE-MNs before and after 10 and 30 min of UV-C exposure. In both exposure conditions, no significant changes were observed in either the slope or the standard potential. A paired t-test assessing slopes before and after treatment indicated no significant alterations for both exposure times, 10 min ($p = 0.82$, $n = 5$) and 30 min ($p = 0.39$, $n = 5$). Regarding the E^0 , slightly larger differences were observed after 30 min of exposure (Change % = 2.03 ± 4.9 , $n = 6$) compared to 10 min (Change % = 0.67 ± 5.52 , $n = 5$). These changes were not statistically significant ($p = 0.36$, $n = 5$). Overall, the results demonstrated that the performance of the WE-MN sensors was preserved following UV-C treatment.

Then, we evaluated the complete patch (i.e., WE-MN + RE-MN). While no significant differences were observed in the calibration slopes, a notable shift in standard potential was detected even after the shorter UV exposure (mean Change % $-4.14 \pm 4.62\%$, $p = 0.02$, $n = 10$) (Figure S8). These results indicated that the RE-MN is particularly susceptible to UV-induced degradation, likely due to alterations in the Ag/AgCl layer affecting the generated reference potential. Considering these findings, one way to minimize any error caused by the calibration shift is to use single-point approach for correction, as previously described in the literature.²⁶ Nevertheless, because this process involves contamination risk during handling, it should only be considered when no contamination can be guaranteed or when fast correction is required.

Subsequently, we explored a double-UV irradiation approach to compensate for any shift caused in the reference electrode potential. This idea was inspired by previous observations in which photochemical changes of Ag/AgCl films are most pronounced upon an initial light exposure, remaining then constant.^{41,42} Accordingly, the double-sterilization protocol comprised two successive UV-C irradiation cycles (“cycle 1” and “cycle 2”) applied under identical conditions with durations of either 5 or 15 min each (total exposure of 10 or 30 min; see Table 1). First, it was aimed to confirm that the calibration remains stable after subsequent irradiation cycles, enabling a practical workflow for pH measurements in real samples: Irradiation (cycle 1)—calibration—irradiation (cycle 2)—measurements. The underlying hypothesis is that the first irradiation cycle causes certain alterations that modify the provided reference potential in the RE but that the change is negligible in the calibration graph with successive irradiation cycles.

Figure 3a and Figure 3b show a representative example of the calibration graphs observed for a pH-MN patch subjected to two consecutive UV-C irradiation cycles. No substantial variations were observed after the second cycle in comparison to the first one (Change % < 1% for the slope and intercept), highlighting that subsequent UV-C cycles produced no significant changes in the RE-MN potential. A similar absence of E^0 shift was consistently observed across independently prepared patches exposed to the same conditions (Change % $-0.55\% \pm 1.48\%$, $p = 0.419$, $n = 5$). These results indicated that the calibration obtained after cycle 1 can be employed for subsequent measurements after cycle 2.

To assess the practical validity of using the calibration graph after the first irradiation to provide accurate pH values in real samples, pH measurements were carried out in known samples with pHs of 5.5 and 8.0, and both calibrations (before UV

irradiation and after the first UV cycle) were used to calculate the pH of the samples. The results obtained from each calibration were compared with the expected pH values provided by a commercial pH meter. When applying the pre-irradiation calibration, Percent differences (Diff. %) between MN-derived pH values and those obtained with the reference commercial pH-meter across the tested standard solutions and exposure conditions ranged from 3.6% to 11.4% (see Table S2). By contrast, using the calibration after cycle 1 reduced the Diff. to 0.4–3.9%, indicating improved accuracy after the initial irradiation. According to these results, it is possible to use the proposed scheme of irradiation–calibration–irradiation–measurements to compensate the initial UV-induced E^0 shift and ensure measurements accuracy. However, as it has been stated before, if the absence of contamination can be ensured, an alternative protocol based on a single-point calibration prior to sensor use, without a second round of UV exposure, may be also applied.

Moreover, the double-irradiation protocol was found to do not impair the reversibility of the MN patch, which maintained consistent responses across repeated measurements. This was evaluated using standard buffer solutions ranging from pH 5 to 8, effectively covering the full range of expected physiological and pathological cases (Figure 3c). The sensor exhibited slightly greater variation compared to the untreated condition, with RSD% of 2.1% for the slope and 5.2% for the intercept.

Microbiological Assessment of Treatment Efficacy on Microneedle Patches. After confirming that ethanol and UV-C treatments did not compromise the analytical performance or structural integrity of the MN-based sensors, the microbiological efficacy of the applied treatments on the entire patch under both practical and regulatory conditions was assessed. We performed two complementary microbial inactivation efficacy tests: (i) under standard-use conditions, which simulates contamination through routine handling (e.g., contact with gloved or bare hands), and (ii) under high microbial load conditions, which involves intentional contamination of the sensor surface with high microbial loads to mimic worst-case scenarios used in disinfection and sterilization validation protocols.

In the first case, standard-use condition test, sensors were incubated directly in sterile culture media at 37 °C without intentional bacterial inoculation, simulating typical microbial exposure resulting from routine handling and environmental contact (Figure 4a). This approach aimed to determine whether the proposed methods effectively eliminate incidental contamination that may occur under real-world operational conditions. Luria Broth (LB) medium was selected due to its widespread use as a general-purpose bacterial growth medium. Its nutrient-rich composition supports the cultivation of a broad range of bacteria, including both Gram-positive and Gram-negative species. This versatility makes LB an appropriate choice for microbiological studies aiming to simulate contamination scenarios commonly encountered on human skin, such as bacteria typically found on hands.^{43,44} Consequently, LB medium serves as a representative environment to evaluate treatments efficacy by assessing the ability of microneedle sensors to eliminate diverse microbial contaminants. The presence of bacterial colonies indicates the disinfection failure.

The qualitative results indicated that both methods (i.e., ethanol and UV-C) are effective in eliminating bacteria from the sensor after typical handling, with 5 min of exposure being sufficient (Table 2). The absence of observable bacterial growth over 24, 48, and 72 h in the sensor culture highlights the

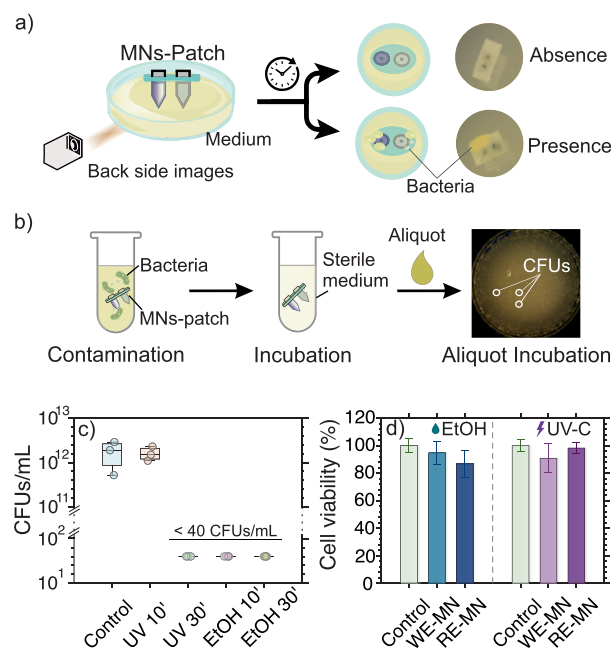


Figure 4. Microbiological assessment and biocompatibility evaluation of MN sensors after treatment. (a) Diagram of the workflow used for the qualitative sterilization test under standard-use contamination conditions. (b) Diagram of the workflow used for the quantitative microbial test under high microbial load conditions. (c) Quantification of microbial contamination (CFUs/mL) recovered from MN sensors after both disinfection protocols. (d) Cell viability assay of MN sensor components (WE-MN and RE-MN) after ethanol (left) and UV-C (right) treatment, compared to untreated controls.

significant effectiveness of the sterilization processes. Pictures of the cultures at these time points for ethanol and UV-C treatments are shown in Figures S9 and S10, respectively. The use of a positive control, an untreated sensor, further confirmed that the observed results are due to the effective sterilization process and not related to other experimental variables such as electrode material, cultivation conditions, etc.

In the high microbial load condition testing, sensors were intentionally contaminated by incubation in *Escherichia coli*-inoculated media at 37 °C for 24 h, in accordance with the criteria of regulatory institutions (FDA and EMA) for high-level disinfection validation.^{45,46} Following contamination, the sensors received the appropriate treatment procedure, being

Table 2. Qualitative Assessment of Selected Treatment Effectiveness for pH-MN Sensors under Standard-Use Conditions: Presence (+) or Absence (−) of Visible Microbial Growth

sterilization method	exposure time, min	24 h	incubation time 48 h	72 h
EtOH 70%	0	+	+	+
	5	−	−	−
	10	−	−	−
	15	−	−	−
	30	−	−	−
UV-C	0	+	+	+
	5	−	−	−
	10	−	−	−
	15	−	−	−
	30	−	−	−
	60	−	−	−

subsequently placed in new sterile media and incubated overnight at 37 °C to facilitate the regrowth of any surviving bacteria. Untreated sensors served as controls. Culture media were serially diluted, plated on LB agar, and colonies counted after incubation. Figure 4b illustrates a schematic representation of the workflow for this experiment. The results are shown in Figure 4c, revealing a significant bacterial reduction, found to exceed seven orders of magnitude relative to untreated controls, indicating high-level disinfection under these experimental conditions. Notably, 10 min of ethanol exposure was sufficient to significantly reduce bacterial load, while UV-C exposure required longer treatment times to reach comparable levels. This highlights the importance of optimizing disinfection or sterilization parameters to meet regulatory standards. Overall, the disinfection efficiency surpassed the 5–6 log reduction threshold defined by European standards, achieving a ≥ 7 -log reduction against *E. coli* under high-challenge conditions, a level of reduction often associated with sterility assurance benchmarks for vegetative bacteria.^{47–50} This preclinical compatibility evaluation demonstrates that ethanol and UV-C treatments are effective under standard-use and high-microbial-load conditions, supporting the safe and reliable use of microneedle-based sensors in preclinical settings. However, full clinical sterilization validation would require additional testing against a broader panel of microorganisms, including bacterial spores and other highly resistant species (e.g., *Bacillus stearothermophilus*). Furthermore, aspects beyond microbial inactivation, such as validation of post-treatment packaging integrity, assessment of dose uniformity across device geometries, and long-term stability after treatment, would need to be addressed for clinical translation.

Biocompatibility Testing. Post-disinfection biocompatibility testing is essential, as pre-treatment characterization does not account for potential treatment-induced changes: disinfection and sterilization steps may alter chemical composition, surface properties, introduce toxic residues, degrade polymers, or modify surface characteristics, potentially impacting cell viability and tissue compatibility. Indeed, regulatory standards mandate post-sterilization biocompatibility assessment to ensure no adverse effects arise during long-term use.⁸ Thus, biocompatibility testing was evaluated for both WE-MN and RE-MN. For the WE-MN, previous studies have reported that ion-selective membranes may present components' leaching after prolonged exposure to cell culture medium (i.e., 24–36 h), potentially leading to cytotoxic effects in the case of certain types of ionophores.⁶ Cell viability was quantified using the MTT assay. Both procedures (ethanol and UV-C) reliably maintained viability over 70% under the experimental conditions, satisfying established biocompatibility standards (Figure 4d). The results demonstrated that MNs sensors can maintain contact with tissue for up to 24 h without compromising cell viability. While these results confirm biocompatibility within the 24 h exposure window, potential long-term effects (i.e., days, weeks) such as membrane component leaching (e.g., plasticizers and ionophores), metal ion leaching of the RE-MN or material changes after repeated disinfection warrant further investigation.

Assessment of Intradermal pH Sensing Performance after MN Sensor Treatment. To evaluate the suitability of the treated pH-MN sensors for accurate intradermal pH measurement, we performed *ex vivo* transdermal sensing using rat skin samples. In essence, the study was conceived to confirm that the high-level disinfection does not compromise the mechanical integrity of the MNs, and that analytical performance remains

reliable to provide reliable transdermal pH measurements. Skin samples were conditioned in buffered solutions of known pH for 24 h at 4 °C, then mounted in a custom 3D-printed holder for measurements with MN patches sterilized with the established protocols based on ethanol and UV-C. After 24 h, the pH inside the skin agreed to that fixed in the soaking solution (see Table S3 in the Supporting Information).²⁸ More information regarding *ex vivo* assay can be found in the Supporting Information. A scheme, and real photographs of the procedure are shown in the Figure 5a and Figure 5b, respectively. Importantly, for validation purposes, a small incision was made in the skin to insert a commercial micro pH meter into it.

For ethanol-based disinfection, the sensors were calibrated after fabrication and conditioning, then expose to ethanol, and used directly for intradermal measurements. In contrast, UV-treated sensors followed a protocol consisting of an initial irradiation (cycle 1), calibration, second irradiation step (cycle 2), and then *ex vivo* measurements. Notably, for UV-C sterilization, two exposure durations were assessed: 10 min (sufficient for normal-use conditions) and 30 min (aligned with regulatory requirements).

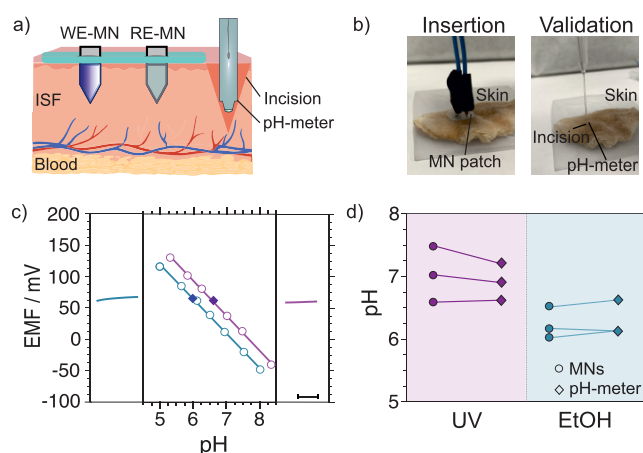


Figure 5. *Ex vivo* intradermal pH sensing using treated MN sensors. (a) Scheme of the *ex vivo* setup showing insertion of MN patches into the skin and the measurement with the micro pH-meter through a small incision. (b) Photographs showing the insertion of the MN patch into *ex vivo* tissue (left) and the commercial pH-meter (right). (c) Determination of *ex vivo* pH. The final potential from the real-time potentiometric response in rat skin (traces on the sides, scale bar = 30 s) is extrapolated onto the corresponding external calibration curve (lines with open circles) to obtain the final pH value (solid diamonds). Data is shown for sensors treated with EtOH (blue) and UV-C (purple). (d) Plot comparing the pH values obtained from the MN sensors (circles) and the reference pH meter (diamonds) for the same *ex vivo* tissue samples, grouped by methods (UV-C (purple) and EtOH (blue)). The connected lines pair the measurements for each sample.

Table 3. Comparison of Intradermal pH Values Obtained with Treated pH Microneedle Sensors and a Commercial pH Meter

treatment	skin sample	pH (MN patch)	pH (micro-pH meter)	diff. (%)
UV (10')	#1	patch 1 7.02 ± 0.11	6.91 ± 0.02	2.0
	#2	patch 2 7.48 ± 0.03	7.22 ± 0.02	4.0
UV (30')	#3	patch 1 6.59 ± 0.11	6.62 ± 0.02	2.0
	#4	patch 2 6.17 ± 0.17	6.13 ± 0.02	0.7
EtOH (10')	#4	patch 2 6.02 ± 0.04	6.13 ± 0.02	1.8
	#3	patch 3 6.51 ± 0.04	6.62 ± 0.02	1.7

Transdermal potentiometric signals were recorded for approximately 1 min, and pH values were determined by extrapolating the average potentials using the respective external calibration (see Figure 5c for an example of the observed readout). The results are collected in Table 3, revealing comparable performance across both strategies. Intradermal pH values measured with the high-level disinfected MN patches were in close agreement with reference values obtained with the commercial pH meter, with Diff.% of <5%. For UV-C, both exposure times presented low error, ranging from 2% to 4%, indicating that prolonged UV exposure does not substantially compromise sensor accuracy. Similarly, ethanol-treated sensors demonstrated excellent performance, with Diff. <2%, suggesting minimal impact on calibration or sensitivity. In addition, a graphical representation of a comparison of pH measurements provided by the MN patch and the pH meter is displayed in Figure 5d. This plot displays the pH values obtained from the treated MN patches (circles) alongside those from the reference pH meter (diamonds) for the same *ex vivo* tissue samples, under both UV and ethanol (EtOH) protocols. The close vertical alignment of each paired data point demonstrates good agreement between the two methods. A mean difference between the two methods close to 0 pH units (i.e., 0.03) was observed (Figure S11a). In addition, excellent agreement was observed between both methods, with a Pearson correlation coefficient of 0.99 and a statistically significant positive correlation (Figure S11b). It should be noted that, as this is a preclinical study, the sample size is limited, and validation in a larger number of subjects will be required for translation.

Overall, these results confirm that the proposed protocols do not compromise the sensor's accuracy, validating its reliability for intradermal pH measurements.

CONCLUSIONS

This work systematically evaluates common methods employed for disinfection and sterilization of potentiometric microneedle (MN) patches, addressing a critical gap for their evaluation in preclinical research settings. Using a pH-selective MN patch of potentiometric wearable sensors as a representative model, we evaluated easily implementable disinfection and sterilization methods, such as ethanol, UV-C, and autoclaving. First, we demonstrated that ethanol disinfection did not compromise the sensor's analytical performance or structural integrity even at prolonged exposure times (30 min). Similar results were obtained for UV-C, with a small adjustment to the treatment protocol. To ensure consistent sensor performance, a minor protocol adjustment by implementing a two-cycle UV-C procedure effectively mitigated the RE-MN potential shift. In contrast, autoclaving was found not to be compatible with the tested MN patch architecture and polymeric membrane composition, as it severely compromised both structural integrity and electrochemical function. Complementary microbiological assays showed that EtOH and UV-C treatments efficiently removed bacterial contamination under both standard-use and high microbial load conditions, meeting preclinical safety requirements. In parallel, no detrimental changes in cell viability were observed before and after treatment, indicating that these procedures do not compromise patch biocompatibility and remain suitable for prolonged tissue contact. Finally, to confirm transdermal applicability, treated patches were used to measure pH on *ex vivo* rat skin, showing high agreement with values obtained using a commercial micro-pH meter as a reference method. Altogether, these results establish EtOH and

UV-C, when UV-C is applied with a two-cycle protocol, as practical, performance-preserving approaches for microneedle-based sensors, supporting their safe and reliable use in preclinical research settings. This validation framework can guide compatibility assessments beyond MN-based potentiometric patches to other potentiometric microneedle sensors employing different ionophores and polymeric membrane compositions and may also serve as a starting point for evaluating sterilization and disinfection compatibility in other microneedle-based sensing platforms.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acssensors.5c04039>.

Experimental details, statistical methods definitions, CFU results, additional pre/post EtOH and UV-C calibrations, double-irradiation accuracy table, and quantitative *ex vivo* assay (DOCX)

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Notes

The authors declare no competing financial interest.

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