Effective and Sustainable Surface Microbial Control using Electric field treatment for Global Hygiene and Industrial Anti-biofouling

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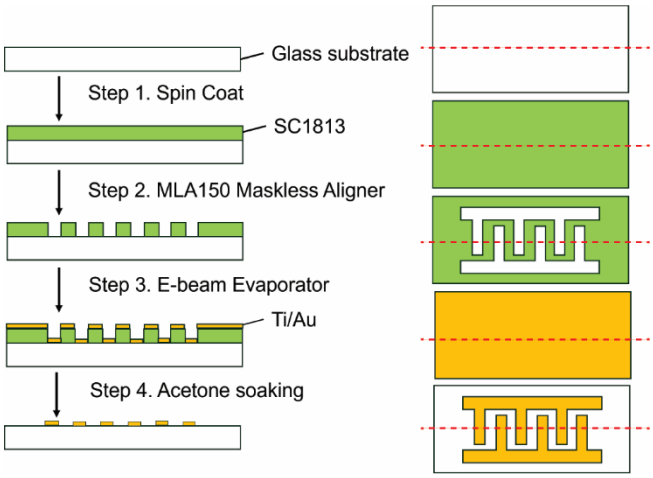

Figure S1. The graphical illustration of fabrication process at the top and cross-sectional view. The left is the cross-sectional view at that dashed red line in the top view (right).

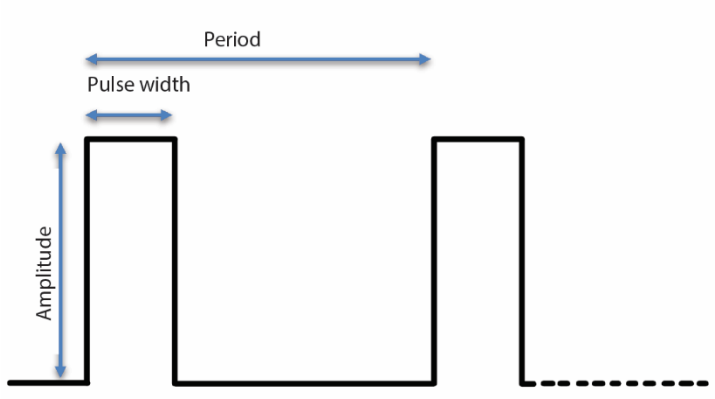

Figure S2. The graphical illustration of the parameters of applied electric pulses. The electrical parameters applied in this study are: amplitude varied from 7 V to 30 V with fixed pulse width of 2 μs and period of 2 ms. The total number of pulses applied in one treatment was 10^4 to ensure the effective treatment time of 20 ms and total treatment time of 20 s.

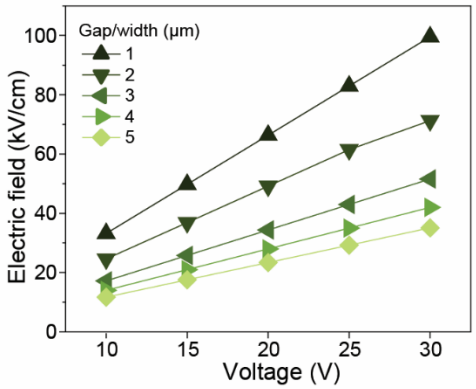

Figure S3. Effects of gap/width of electrode fingers and voltage on the electric field strengths on MIDEs with COMSOL. The linear relationship between the voltage and electric field strength is observed.

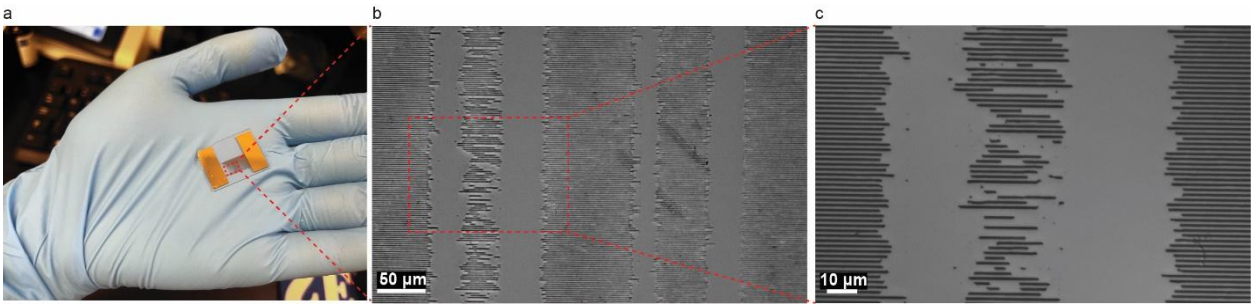

Figure S4. The images of failed 1 µm gap/width MIDEs. a. Digital image of MIDEs (1 µm gap/width and 10^4 µm length). The large yellow area represents the electrode pads and the MIDEs are between them. **b.**, **c**. Zoomed-in microscopic images showing the malfunction of MIDEs structures. Magnified image in red dashed square in a. (b) and further magnified image of red dashed area in b. (c). It is suggested the failure of MIDEs was caused by the inconsistency of photoresist on wafers during the spin coater. The non-uniform photoresist causes the non-uniform pattern acquired after exposure. It will be more significant for small features (1 µm). Specifically, areas with thicker photoresist tend to undergo underexposure, causing residual photoresist to remain on the wafer and gold deposition removal during lift-off.

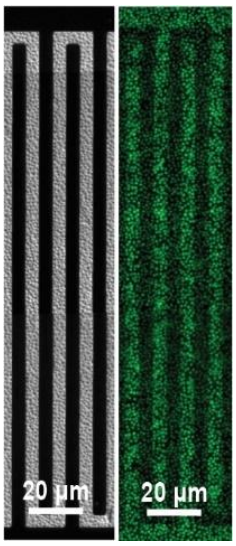

Figure S5. Microscope images of MIDEs with bacterial immobilization. Microscope images representing the successful immobilization of model bacteria on MIDEs (5 µm gap/width and 200 µm length) under DIC (left) and SYTO 9 (right) channels, respectively. The bacterial immobilized above MIDEs electrode fingers were invisible under the DIC channel while visible under the SYTO 9 channel.

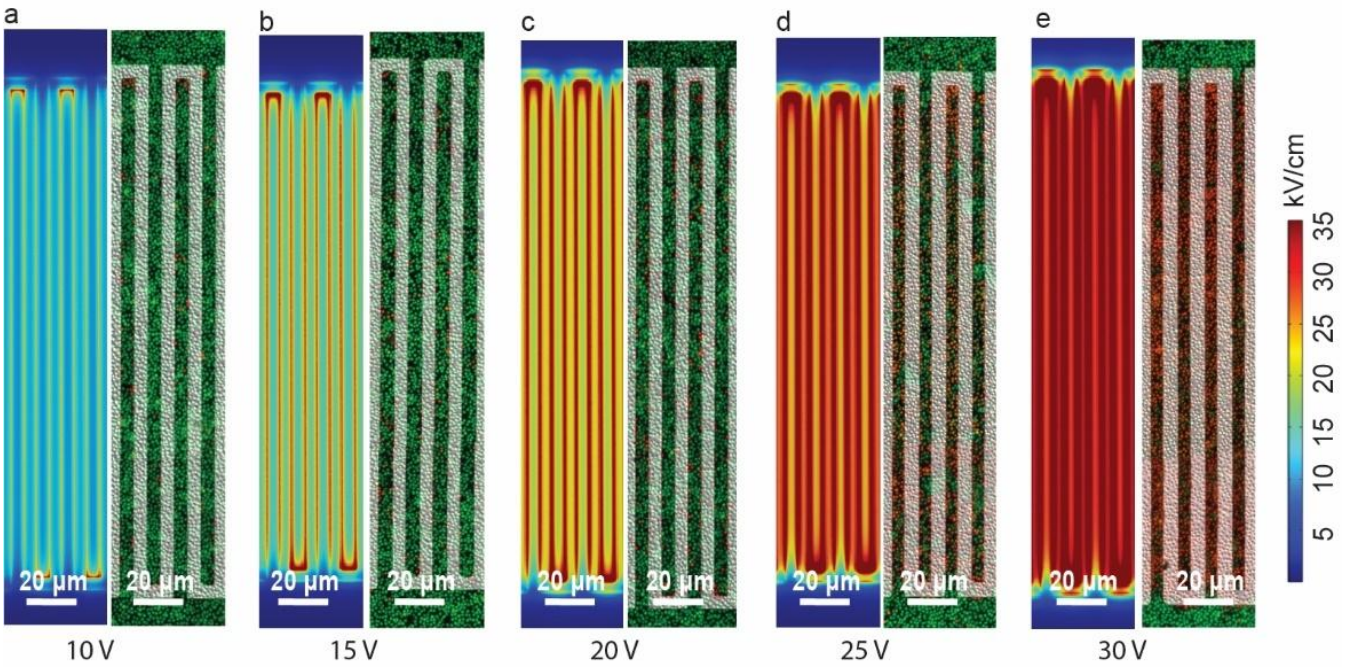

Figure S6. The electric field strength and the bacterial inactivation distribution on MIDEs. a.-e. the electric fields and the bacterial inactivation distribution of MIDEs with 5 µm width/gap and 200 µm length at **(a)** 10 V, **(b)** 15 V, **(c)** 20V, **(d)** 25 V, and **(e)** 30V based on COMSOL. The electric field strength is intensified at the edge of the electrodes and can reach an overall high electric field at a high voltage (25, 30 V). The bacterial inactivation, indicated by red signals, only showed at the edges at a lower voltage (10-20 V) while covering the whole MIDEs surface at a higher voltage (25, 30 V). Their consistency further suggests that the close relationship between electric field strength and bacterial inactivation.

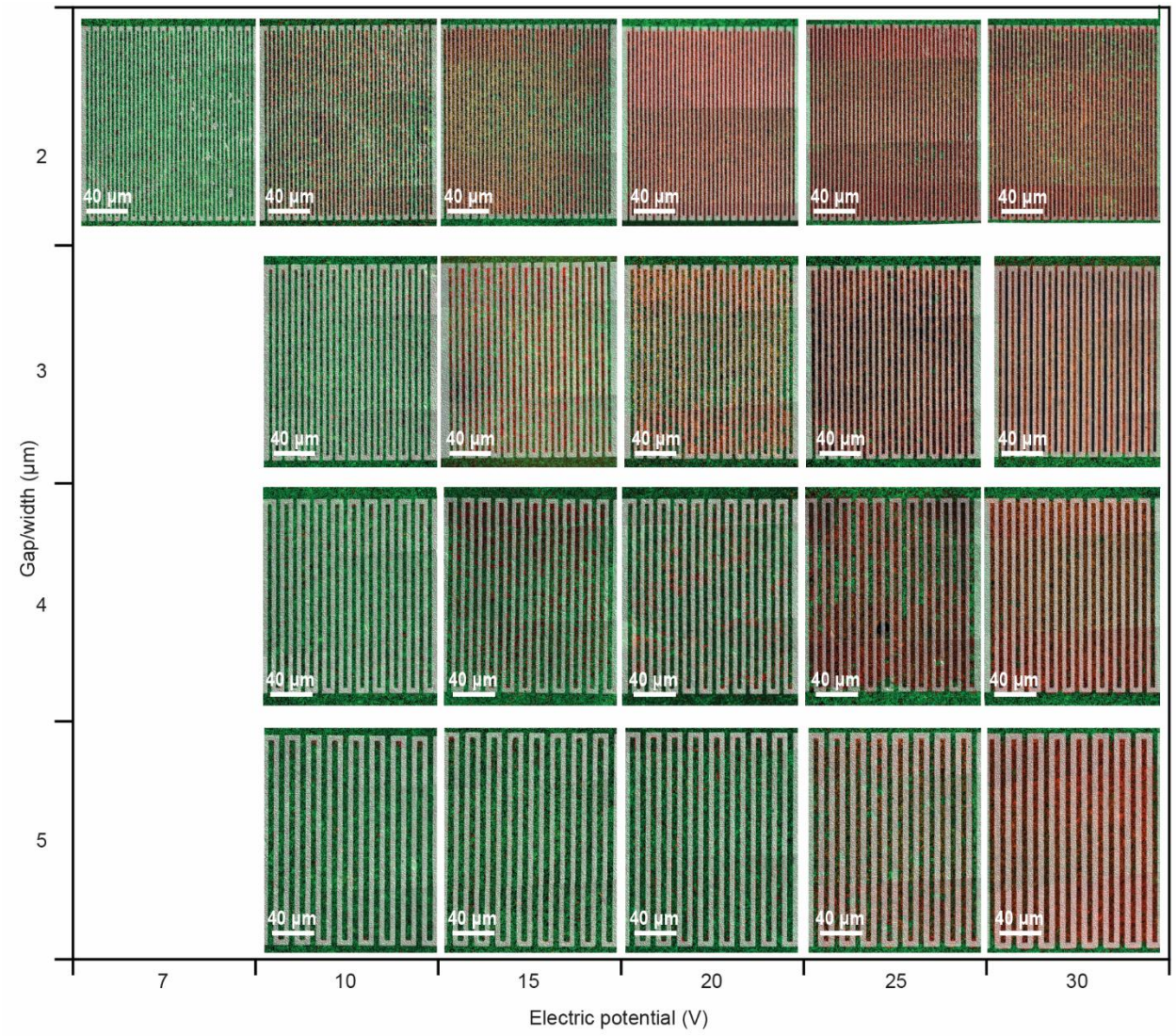

Figure S7. Microscope images of bacterial inactivation under various conditions. Increased voltage contributed to increased red signals indicating increased antimicrobial efficiency. The voltage necessary for bacterial inactivation increased with increased gap/width for MIDEs.

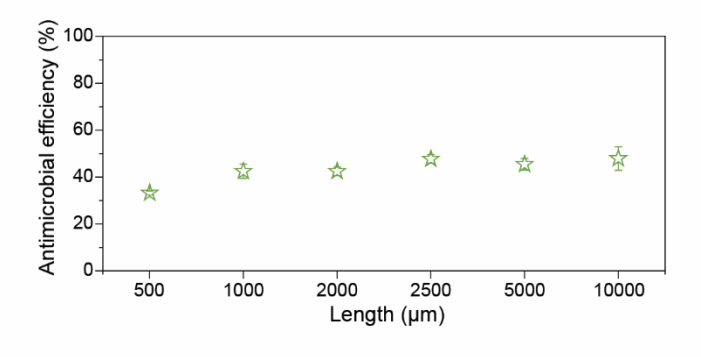

Figure S8. Effect of MIDEs length (5 μm gap/width, 50 to 10^4 μm) on antimicrobial efficiency at 20 V. Lower voltage chosen to emphasize length effects and avoid antimicrobial efficiency saturation at 30 V. Length variation from 500 μm to 10^4 μm pose negligible influences on the antimicrobial efficiency.

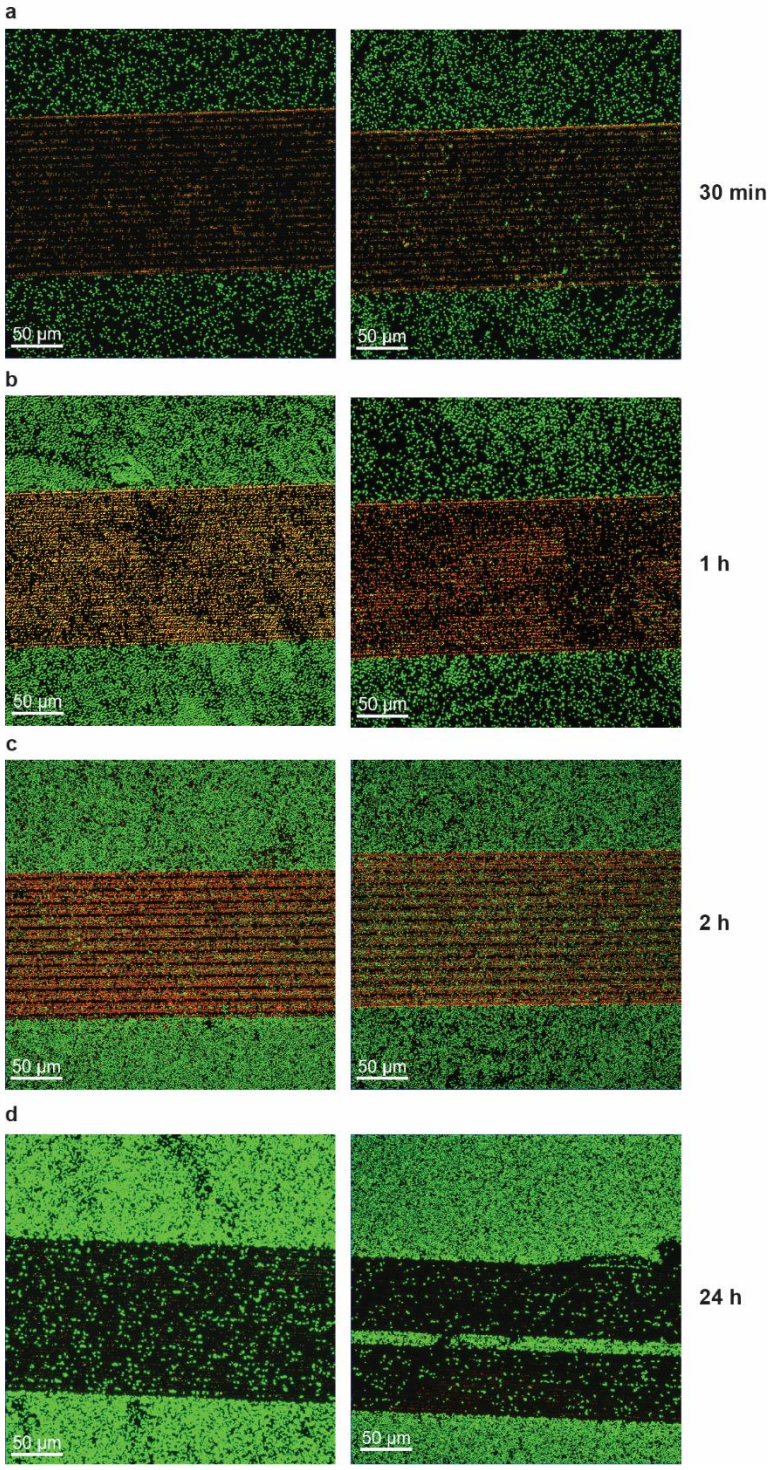

Figure S9. CLSM images of biofilms on MIDEs after cultivating for different time. Images are acquired after cultivating for **(a)** 30 min, **(b)** 1 h, **(c)** 2 h, and **(d)** 24 h. Microbes are inactivated on MIDEs showing red signals and detached after longer cultivation (24 h), while constantly grow on control areas, glass substratum in these CLSM images, indicated by the intensifier green fluorescence.

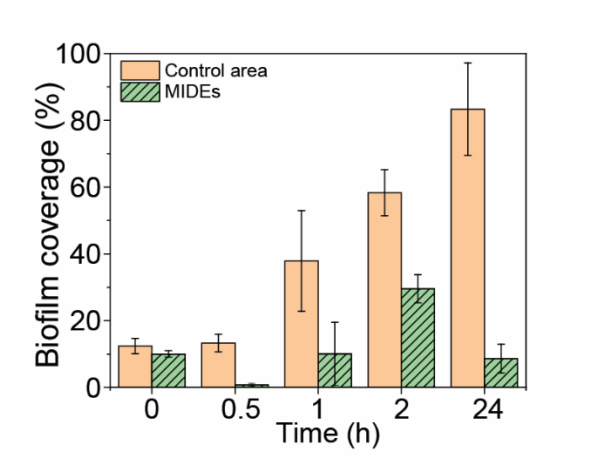

Figure S10. Quantified time evolution of biofilm coverage on MIDEs and control areas. Error bars show the standard deviation from 10 randomly selected images from the device. Biofilm coverage is quantified as the percentage of fluorescence pixels on the substratum with COMSTAT 2.1.

Table S1. The detailed parameters of IDEs pattern

Width	2, 3, 4, and 5 μm
Gap	2, 3, 4, and 5 μm
Length	200 μm
Depth	10 nm Ti, 100 nm Au

Text S1. Detailed description of the addition order of fluorescence dyes

SYTO 9 stain is a green-fluorescent stain that is permeant to microbial membranes (https://www.thermofisher.com/us/en/home/life-science/cell-analysis/fluorophores/syto-9.html). It is powerful for bacterial assay since it stains the DNA of both live and dead bacteria. SYTO 9 is added before electric field treatment to count the total number of bacteria deposited on the MIDEs.

Propidium iodide (PI) is a red-fluorescent stain that does not permeant to intact membranes of live cells, and thus it is commonly used to detect membrane-demised and dead cells in a population (https://www.thermofisher.com/us/en/home/life-science/cell-analysis/fluorophores/propidium-iodide.html). PI staining can be induced right after electric field treatment. However, it is reported that reversible and irreversible membrane damage both contributed to the PI staining, but the reversible one should not be counted for bacterial inactivation.[1] To improve the accuracy of antimicrobial efficiency quantification, PI was added 10 min after treatment to exclude the bacteria underwent reversible membrane damage, considering that it usually can be recovered within 2-3 min after treatment.[2] Thus, our calculations of inactivation efficiency is based on the division of PI staining cell counts, indicating the irreversible electroporation, by the SYTO 9 staining cell counts before treatment, indicating the total cell numbers.

For the time-evolution experiments, SYTO 9 and PI were added simultaneously before electric field treatment. This approach will allow us to monitor the PI staining process and quantify the response during experiments.

Text S2. Digital Image processing with MATLAB

The microscopy images acquired in the antimicrobial test were processed with MATLAB (version R2023b, MathWorks). With DIC channel, images were rotated to the orientation where electrode fingers were parallel to the edge of the images. This step was for better cropping the image to exclude the area without MIDEs. The rotated angle was acquired by processing image under DIC channel with low pass gaussian filter to capture the orientation MIDEs patterns. Then, the angle was acquired with Hough transform, and images were rotated in all channels with the corresponding angles. Afterwards, the images were cropped to region of interests (ROIs) to exclude the areas without MIDEs, which were regarded as control areas (electrode pad and glass substrates). Finally, cell numbers were counted with regional max function within ROI under PI and SYTO 9 channels, respectively. The definition of bacterial inactivation efficiency was the percentage of inactivated bacteria (cell counts from reddish signal under PI channel after electric field treatment) to total bacteria (cell counts from green signal under SYTO 9 channel before treatment). The mathematical formula of bacteria inactivation efficiency is represented as:

$$Antibacterial\ \ efficiency\ (\%) = \frac{number\ of\ cell_{PI}}{number\ of\ cell_{SYTO\ 9}} \times 100\%$$

Two major experimental errors during the process can lead to deviations in antimicrobial efficiency from the true value. First, extreme electric field treatment of bacteria can induce bacterial lysis. The lysed cells were non-fluorescent caused by the loss of intracellular molecules.[3] They are a part of bacterial inactivation and are neglected during this quantification process, decreasing the acquired antibacterial efficiency; Second, it is reported that staphylococcus epidermidis is vulnerable to the attachment from the electrostatic force of anode.[4] After electric field treatment, the attraction causes the bacterial overlap under microscope images which will miscount cell number (e.g. 2 or more bacteria overlapped and misread into 1) and also decrease the acquired antibacterial efficiency.

References

1. Wang, T., Brown, D.K. & Xie, X. Operando Investigation of Locally Enhanced Electric Field Treatment (LEEFT) Harnessing Lightning-Rod Effect for Rapid Bacteria Inactivation. *Nano Lett* **22**, 860-867 (2022).
2. Jarin, M., Wang, T. & Xie, X. Operando investigation of the synergistic effect of electric field treatment and copper for bacteria inactivation. *Nat Commun* **15**, 1345 (2024).
3. Krämer, C.E.M., Wiechert, W. & Kohlheyer, D. Time-resolved, single-cell analysis of induced and programmed cell death via non-invasive propidium iodide and counterstain perfusion. *Scientific Reports* **6** (2016).
4. Lou, Y., Thebault, P., Burel, F. & Kébir, N. Antibacterial properties of metal and PDMS surfaces under weak electric fields. *Surface and Coatings Technology* **394** (2020).