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Published in:
LWT

Link to article, DOI:
10.1016/j.lwt.2024.117158

Publication date:
2024

Document Version
Publisher's PDF, also known as Version of record

LWT - Food Science and Technology 214 (2024) 117158

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

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ARTICLE INFO

Keywords:
Plasma
Microbiology
Food safety
Pathogen control
Escherichia coli

ABSTRACT

There is a constant demand for innovative technologies that can safely reduce spoilage and pathogenic microorganisms in food without compromising the taste, safety or quality of the product. Aerosols of plasma-activated water (PAW) generated from distilled water may serve as innovative and alternative surface disinfectants in food and veterinary production systems. This study examined the effectiveness of a PAW aerosol-producing device in reducing *Escherichia coli* dried onto metal discs. These metal discs were placed in a 1 m^3 chamber and sprayed with the PAW aerosol for 15 s or 1 min and from a 50 or 70 cm distance. Metal discs placed in a Petri dish protected from direct spray served as a reference control for the treated metal discs. A total of 31 metal discs were tested in 10 trials, and only 2 demonstrated a significant reduction (>4 $\log_{10}$) of *E. coli*. Contrary to previous studies, where immersion of food into PAW indicated a potential for disinfection in the food processing industry, the results indicate the limitations of PAW sprayed as an aerosol for disinfection of surfaces. Further studies would be needed to establish the capacity of PAW to disinfect depending on the application and target.

1. Introduction

The modern food industry faces two significant challenges that have substantial economic and social impacts: foodborne diseases and food waste (World Health Organization, 2015; Bradford et al., 2018). Currently, food production relies on chemical agents and thermal control to ensure food safety and enhance shelf-life; however, these approaches may adversely affect the quality characteristics of the food (Knorr et al., 2011). Organic food production systems especially need effective decontamination methods that do not involve chemical agents, as their use violates organic principles and legislation (European Commission, 2008). Therefore, innovative decontamination technologies that can reduce pathogenic and spoilage pathogens in food production are urgently needed (Han et al., 2023).

Plasma-activated water (PAW) technology involves treating water with non-thermal plasma created by applying an electric charge to atmospheric air, resulting in a mix of ionised gas. This ionised gas includes electric fields, ultraviolet photons, and charged particles (Zhao et al., 2019. When this plasma interacts with water and atmospheric air molecules, it creates reactive oxygen and nitrogen species (RONS) (Ma et al., 2015; Moisan et al., 2002). The physical properties of water can change following plasma activation, leading to decreased pH and surface tension and increased electrical conductivity and oxidation-reduction potential (Ma et al., 2015; Shaji et al., 2023; Tian et al., 2014). When PAW is sprayed, it forms aerosols that further interact with air, enhancing the generation and effectiveness of RONS for disinfection purposes. A variety of RONS can be contained in PAW, such as hydrogen peroxide, ozone, and hydroxyl radicals (Khlyustova et al., 2019). Several studies concluded that RONS can exert an antimicrobial effect by disrupting the cellular structures, causing bacterial inactivation (Darmanin et al., 2020; Lin et al., 2020; Thirumdas et al., 2018; Zhang et al., 2013). Immersion tests have demonstrated a reduction in bacterial load when food is submerged in distilled water treated with PAW (Hadinoto et al., 2023a, 2023b; Ma et al., 2015; Perinban et al., 2022). Based on water and plasma, PAW treatment minimises harmful and persistent chemicals and constitutes a novel, eco-friendly disinfectant. However, it does generate reactive oxygen and nitrogen species (RONS) that require careful management (Thirumdas et al., 2018).

One of the most common bacterial foodborne pathogens is *Campylobacter* spp. (WHO, 2015), and poultry is considered the most

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https://doi.org/10.1016/j.lwt.2024.117158
Received 12 July 2024; Received in revised form 25 September 2024; Accepted 5 December 2024
Available online 5 December 2024

common source. A higher flock prevalence of *Campylobacter* is commonly observed for free-range and organic broilers than conventional broilers due to a different management system, where e.g. access to outdoor areas impairs the biosecurity options (Lassen et al., 2023). Despite thorough cleaning between broiler flocks, *Campylobacter* can persist in the environment, enabling contamination between flocks (EFSA 2020). Moreover, control is particularly challenging for organic farms, where the disinfection options for a stable environment are limited to certain non-chemical disinfectants approved for organic production systems. Considering that PAW uses water as the only input component and RONS has a very short half-life, the PAW device represents a promising innovation with the potential to work as a surface disinfectant for organic production systems.

A recent innovation named SCORPIUS (PAW aerosol-producing device) (IPLASMA-DE GMBH, Berlin, Germany) generates PAW from distilled water to disinfect surfaces. According to the manufacturer, the primary antimicrobial agents in the 10–50-μm aerosols generated by the PAW device are ROS, RONS, OH, and nitrogen compounds, which have the potential to damage the membrane of bacteria (Potapov, 2021). The testing of disinfection efficacy was a 99.99% reduction of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Salmonella* Typhimurium was reported for an application time of 1–2 s/m^3 followed by 60 min of contact time and was performed according to the Russian Guideline P 4.2.2643–10 (Guideline P 4.2.2643-10, 2010; Potapov, 2021).

The standardised methods for evaluating the efficacy of biocides for the disinfection of surfaces in the EU involve the fixation of the target bacteria onto metal discs (European Committee for Standardization, 2019a, 2019b, 2020). Although *Campylobacter* was the organism of interest, its sensitivity to drying may give rise to misleading results. For this reason, *E. coli* is a more suitable test bacterium for this purpose as it is more resilient to drying on surfaces (Louis et al., 1994; Pispan et al., 2013; Stasic et al., 2012). In addition, *E. coli* is one of the obligatory test organisms for surface disinfection testing, while *Campylobacter* spp. Are not (European Committee for Standardization, 2019a, 2019b, 2020).

To investigate the potential of the application of a PAW aerosol-producing device as an innovative method for surface disinfection in the food and veterinary industry, the aim of the study was to test if the Scorpius device can reduce *E. coli* on metal discs by methods equivalent to the European standards (EN) used for assessment of biocides.

2. Materials and methods

2.1. Plasma-activated water aerosol-producing device

Two identical Scorpius devices (IPLASMA-DE GMBH, Berlin, Germany), based on a sliding discharge mechanism, were used in the experiment. In these devices, plasma is created between paired divergent electrodes separated by 8.5–14 mm, with voltage pulses of 20 kV amplitude and a repetition frequency of 36 kHz applied through the electrodes. The power released in the discharge is 120 W. Distilled water was passed through a nozzle with a required airflow of 30 L/min to create an aerosol mist with a 30 ml/min flow rate (Aristova et al., 2021).

2.2. Experimental setup for the test of disinfection efficacy

2.2.1. Experimental setup

The experimental protocol for testing the disinfection efficacy of the PAW device was developed based on two European standard procedures: Standard EN 17272:2020 for airborne room disinfection (European Committee for Standardization, 2020) and Standard EN 13697:2019 for surface disinfection (European Committee for Standardization, 2019a, 2019b), with slight modifications. The methacrylate experimental chamber measured 1 m^3 (1.2 m × 0.9 m x 0.93 m) and had a custom-made opening, allowing the nozzle of the PAW device to spray into the experimental chamber (Fig. 1).

To test the efficacy of the treatment, metal discs with *E. coli* (see 2.2.2) were positioned inside the experimental chamber in front of the nozzle of the PAW device at a 50 or 70 cm distance and a height of 45 cm above the chamber floor. The metal discs were mounted on a laboratory

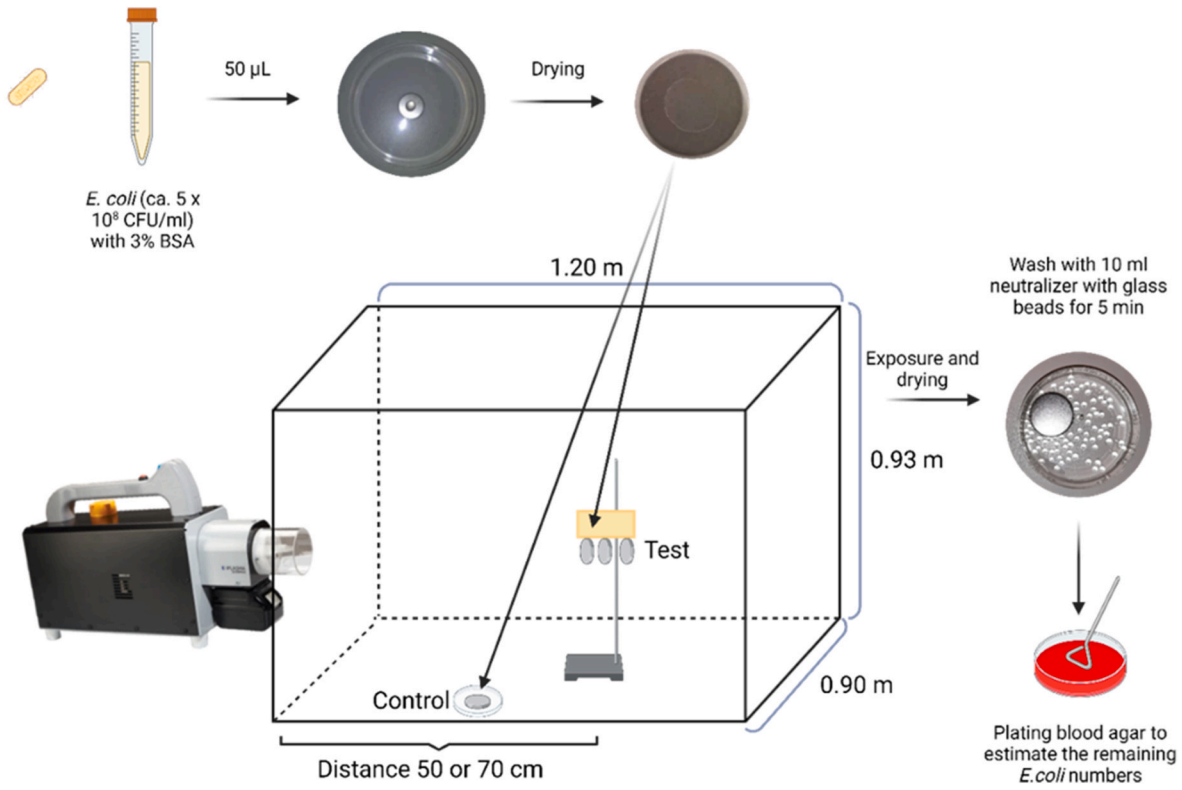

Fig. 1. Experimental set-up for testing the disinfection efficacy of the PAW device. Created with https://biorender.com

stand using adhesive tape and magnets, with a distance of 10 cm between the individual discs.

A metal disc with *E. coli* was placed in a closed Petri dish to serve as a reference control and was positioned on the floor of the experimental chamber, not exposed to PAW aerosols (Fig. 1; Control). For comparison of disinfection efficacy, two additional metal discs with *E. coli* were treated with either 70% ethanol or a commercial hydrogen peroxide disinfectant (Oxivir Excel® Foam, Diversey, Utrecht) at a weight per cent of 0.1–1 for 1 min. The treated discs were placed outside the chamber to avoid exposure to PAW aerosols.

A series of 10 tests were performed varying the spraying distance (50 or 70 cm), duration of spraying (15 s or 1 min) and drying time (60 min), types of water utilised (distilled water from the laboratory or demineralised (Borup, Vejle, Denmark)), and the PAW devices (Table 1). The distance was half the manufacturer's recommended maximum spraying distance (50 cm) and 20 cm from the back wall of the testing chamber (70 cm). The duration of the spraying time was selected according to the manufacturer's recommendation (15 s), and the minimum exposure time (1 min) was according to EN 13697 (European Committee for Standardization, 2019a, 2019b). After the application of PAW and subsequent drying time, the metal discs were retrieved to enumerate the remaining *E. coli*.

2.2.2. Preparation of metal discs for test of disinfection efficacy

A frozen stock culture of *Escherichia coli* ATCC 10536 was streaked onto blood agar (BA) plates (Blood Agar Plates, SSI Diagnostica A/S, Hillerød, Denmark) and incubated at ambient temperature overnight. One ml buffered peptone water (BPW, Oxoid Ltd., Basingstoke, United Kingdom) was added to the BA plate, and the colonies were dissolved by mixing with a Drigalsky spatula. The resulting suspension was extracted into a 10 ml tube using a pipette and then vortexed thoroughly. The *E. coli* suspension was diluted until reaching an optical density (OD; Spectrometer UV mini-1240, Shimadzu Europe, Duisburg, Germany) of 0.6, equivalent to approx. 1.0 x 10^9 colony-forming units (CFU/ml). *E. coli* in the stock suspension was enumerated by preparation of a 10-fold dilutions series in BPW and subsequent plate-spreading of 0.1 ml of appropriate dilutions onto BA plates, followed by incubation at 37 °C overnight.

A bacterial "working culture" was prepared by mixing 1 ml of the stock suspension with 1 ml of bovine serum albumin (BSA) 6 % to reach a concentration of 3.0 g/L BSA to simulate dirty conditions. Fifty microliters of the working culture were added to the centre of a 2 cm diameter and 1.4 mm thick sterile stainless-steel discs (P/N 304-207 L, Pegen Industries Inc., Canada) placed in a Petri dish. After thoroughly spreading the culture, the discs were air dried for a maximum of 45 min within an incubator at 37 °C, with the lid slightly opened to facilitate gradual evaporation of the working culture. After drying, the metal discs with *E. coli* (test and controls) were placed in the experimental chamber as described in 2.2.1.

In addition to the test and control metal discs, a neutraliser control was performed to investigate potential toxicity to *E. coli*. The neutraliser control was a metal disc with *E. coli* not treated with PAW aerosols, which was placed in a Petri dish containing 10 ml neutraliser and sterilised glass beads with a diameter of 4 mm, followed by the addition of 0.1 ml distilled water. The metal disc was washed with distilled water, and the *E. coli* was enumerated as described below in 2.2.3. In addition, a method validation test was performed twice to assess whether the neutraliser could effectively neutralise the disinfectant. For this validation, a metal disc with *E. coli* was placed in a Petri dish (10 cm diameter) with 10 ml neutraliser and glass beads, followed by the addition of 0.1 ml ethanol 70%. The metal disc was washed with distilled water, and the *E. coli* was enumerated as described below in 2.2.3.

2.2.3. Enumeration of E. coli on metal discs

After PAW aerosol exposure, the metal discs were placed in a Petri dish containing 10 ml of neutraliser (Lecithin 3 g/L; polysorbate 80 30 g/L; sodium thiosulfate 5 g/L; L-histidine 1 g/L; saponin 30 g/L dissolved in Maximum Recovery Diluent (Oxoid Ltd., Basingstoke, United Kingdom)) and glass beads. The dried surface of the disc faced downward to ensure contact with the beads, and the Petri dish was shaken for 1 min on a shaker table at approximately 80 rpm. After an additional 4 min, 1 ml was extracted to enumerate *E. coli* as described above (2.2.2). The metal discs were then rinsed with 10 ml of distilled water and transferred to a Petri dish with Trypticase Soy Agar (TSA). The TSA was poured to ensure complete contact with the metal discs, allowing for the assessment of the remaining *E. coli* on the discs. The plates were incubated at 37 °C for 24 h to enumerate *E. coli*.

2.3. Measurement of the physical properties of water

The distilled and demineralised water used for PAW generation (listed as "before"), as well as the generated PAW ("after"), was collected once into a Petri dish and analysed for the following parameters: The salt content (%) and conductivity (μS/cm) were measured using an HQ40D Digital Multimeter Kit with a CDC401-03 conductivity standard electrode (HACH, Iowa, USA), while the pH was measured with the same multimeter kit but equipped with an Intellical PHC101 Laboratory pH Electrode.

2.4. Data management

The *E. coli* reduction by PAW treatment was calculated as the difference in *E. coli* numbers between the exposed test metal disc and the control disc protected from PAW. The same control was used to calculate the reduction effect with ethanol and hydrogen peroxide. A reduction of >4 log 10 was deemed sufficient for a bactericidal effect (European Committee for Standardization, 2019a, 2019b; 2020).

Table 1
Summary of the conditions for testing the disinfection efficacy of PAW on *E. coli* dried on metal discs and the resulting reduction.

Test	Exposure time; Drying time	Distance	Type of water	PAW device	*E. coli* $\log_{10}$ reduction on test discs					Number of discs >4 $\log_{10}$ reduction of *E. coli*/Total number of discs tested
1	1 min; 60 min	50 cm	Distilled	1	5.21	0.08	NA	NA	NA	1/2
2	1 min; 60 min	50 cm	Distilled	1	5.25	−0.06	0.37	0.02	0,00	1/5
3	1 min; 60 min	50 cm	Distilled	1	0.39	−0.53	0.62	NA	NA	0/3[a]
4	1 min; 60 min	50 cm	Distilled	1	−0.75	−0.44	−0.62	NA	NA	0/3
5	1 min; 60 min	70 cm	Distilled	1	−0.05	0.41	−0.15	NA	NA	0/3
6	15 s; 60 min	50 cm	Distilled	1	0.11	−0.45	0.23	NA	NA	0/3
7	1 min; 60 min	50 cm	Distilled	2	−0.41	−0.63	−0.02	NA	NA	0/3
8	1 min; 60 min	50 cm	Demineralised	2	−0.02	−0.52	−0.18	NA	NA	0/3
9	1 min; 60 min	50 cm	Demineralised	2	−0.59	−0.69	−0.29	NA	NA	0/3
10	15 s; 60 min	50 cm	Demineralised	2	−0.32	−0.92	−0.25	NA	NA	0/3

NA: Not applicable.
[a] In test 3, all discs were placed in proximity, contrary to the other tests where discs were placed within a 10 cm distance.

3. Results

A sufficient bacterial reduction (>4 $\log_{10}$) was observed on 2 of 31 metal discs (Table 1); the exposure time was 1 min with 60 min of drying in both tests. Application time, spraying distance, and type of water did not influence the reduction of *E. coli*. The second PAW device (test 7–10) failed to reproduce the reduction observed with the first PAW device.

The *E. coli* concentrations remaining on the neutraliser control discs were >5 $\log_{10}$ CFU/ml in all the tests (n = 10), indicating the absence of toxicity from the neutraliser. In addition, the method validation showed an *E. coli* concentration >5 $\log_{10}$ CFU/ml (n = 2), verifying that the neutraliser did neutralise the disinfectant.

Disinfection tests with 70% ethanol (n = 10) and weight per cent of 0.1–1 hydrogen peroxide (n = 2) resulted in >5 $\log_{10}$ reduction of *E. coli*.

The physical properties (salt %, conductivity, and pH) of the two water types used for PAW generation are shown in Table 2. Plasma activation increased salt content and conductivity and decreased pH from 4 to 5 to about 3 due to the formation of reactive oxygen and nitrogen species (RONS). These changes highlight the impact of plasma activation on water chemistry, suggesting that RONS significantly influence water acidity and ionic composition.

4. Discussion

This study tested the bactericidal effect of PAW in aerosol form when applied to surfaces. A reduction of >4 $\log_{10}$ is required to claim the bactericidal activity of a surface disinfectant according to DS/EN 17272:2020 and DS/EN 13697:2019 Standards. However, the PAW treatment of *E. coli* under simulated dirty conditions (3% BSA) failed to meet this requirement in the majority of the tests (93%), where hardly any reduction was obtained. The PAW technology and its application for reducing the bacterial load on food have shown some promise in previous studies (Darmanin et al., 2020; Lin et al., 2020; Ma et al., 2015; Thirumdas et al., 2018; Zhang et al., 2013). When lettuce, tomato, or eggs were immersed in PAW-treated water in five experimental setups, the bacterial load was reduced by 4 $\log_{10}$ (Hou et al., 2021; Joshi et al., 2011; Lin et al., 2020; Schnabel et al., 2015; Schnabel et al., 2021). Noticeably, the European standards for immersion require a reduction of >5 $\log_{10}$ (EN 1276:2019) (European Committee for Standardization, 2019a, 2019b), and this was only obtained in three out of the five studies (Hou et al., 2021; Lin et al., 2020; Schnabel et al., 2021). A biocidal effect comparable to that seen in immersion tests has not yet been demonstrated by surface spraying with PAW aerosol. For example, spraying 1 ml of PAW onto fresh-cut pieces of kiwi reduced *Staphylococcus aureus*. However, after eight days of storage, the difference between PAW-treated and non-treated fruits was only 1.8 $\log_{10}$ (Zhao et al., 2019). Although PAW exerts some potential for reducing bacterial loads on surfaces, its disinfection efficacy, when applied as a spray, is in its current stage of development, lower than the level required by biocide standards.

The spraying of PAW as a potential surface disinfectant has previously shown a 4-log reduction in *E. coli* (Burlica et al., 2010). The discrepancy between our results and those of Burlica et al. (2010) may be attributed to differences in, e.g. distance (30 mm) between the plasma device and the target surface, duration of treatment (2 min), and the use of agar plates spiked with bacteria instead of bacteria dried on metal discs. Additionally, they incorporated hydrogen peroxide (H_2O_2) into the water solution, contributing to the reduction of the bacterial load (Burlica et al., 2010). This is, however, not surprising considering the inherent bactericidal properties of H_2O_2 (Hyslop et al., 1995), further implying that observed reductions of bacterial load cannot be solely attributed to PAW when H_2O_2 has been added to the water.

This study observed a notable reduction in *E. coli* on one of five discs in two of the initial tests, but this reduction was not reproducible in the other tests. This inconsistency suggests that the reduction observed initially may have been due to human error or inaccuracies in technique. Consequently, we cannot conclusively determine the efficacy of PAW in reducing *E. coli* based on these preliminary findings. However, we tested our setup by spraying with alcohol and hydrogen peroxide instead of the PAW aerosol, which did show the expected biocide effect. It is possible that passing a water aerosol through a plasma beam is not effective enough in creating enough PAW-charged aerosols. Producing PAW inside the device before releasing it as an aerosol may be more effective. A device failure, e.g., lower power release than expected, cannot be ruled out despite using two functional instruments for PAW generation with fully charged batteries. However, this is unlikely as neither device could reduce the bacteria. We found a slight change in salt content, pH, and conductivity in the water before and after activation with plasma. This indicates some effect of plasma on the chemistry of the water, which was not further evaluated during the study. The experimental set-up in terms of spraying distance and duration was chosen to be half of the maximum distance and the recommended duration according to the manufacturer's instruction and scientific report provided by the manufacturer (Aristova et al., 2021). Therefore, the lack of effect under the manufacturer's guidelines was unexpected, but further exploration of different test conditions may have helped to achieve a higher reduction; however, this is uncertain.

Table 2
Physical properties of water before and after plasma activation.

Water type	Distilled water		Demineralised water	
Plasma activation	Before	After	Before	After
Salt %	0	0.14	0	0.11
Conductivity (μS/cm)	1.9	252	2.54	213.2
pH	4.22	2.94	5.22	3.00

5. Conclusion

The Scorpius device producing PAW aerosol sprayed on a surface as a disinfectant did not demonstrate a consistent measurable bactericidal effect on *E. coli* in an experimental setup adapted from standardised methods for the efficacy testing of biocides. Further optimisation and studies would be needed to clarify the disinfection potential of a PAW technology that uses aerosol in contact with plasma created between paired divergent electrodes.

CRediT authorship contribution statement

Cristina Calvo-Fernandez: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Brian Lassen:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation. **Annette Nygaard Jensen:** Writing – review & editing, Methodology, Investigation. **Nao Takeuchi-Storm:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Funding

This project was part of SafeChicken: Increased Food Safety of Chicken Meat via Methods to Reduce Campylobacter in Broiler Production (Jan 2022–Aug 2025). It was supported by the Grønt Udviklings-og Demonstrations Program (GUDP) under the Ministry of Food, Agriculture, and Fisheries of Denmark.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

Acknowledgement

We extend our gratitude to Plasmax ApS for technical support regarding the SCORPIUS (PAW device) and to Pia Engelsmann and Resadije Idrizi for their contributions to the laboratory work.

Data availability

No data was used for the research described in the article.

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