

Article

Treatment and Cleaning of Contact Lenses with Plasma-Activated Solutions

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Abstract

The occurrence of contact lens complications caused by inadequate cleaning of the lenses using “All-in-One” contact lens cleaning solutions (CLCSs) represents a medically relevant problem worldwide. This study explores the potential of cold atmospheric plasma (CAP) to enhance the efficacy of CLCSs and address complications from inadequate lens hygiene. It was examined whether exposure to CAP for 1–24 h could boost the antibacterial effects of CLCSs and other solutions, including Milli-Q water (M-QW), physiological saline (NaCl), and Dulbecco’s Phosphate Buffered Saline (DPBS). Additionally, the stability of reactive oxygen and nitrogen species (RONS) and their impact on pH immediately after treatment and over 1–4 weeks was assessed. Furthermore, the cleaning efficacy of plasma-activated solutions (PASs) was tested on lipid-coated silicone hydrogel lenses. Results showed that CAP increased RONS concentrations immediately, with elevated levels persisting over time. While no significant improved antibacterial effect was observed against *Escherichia coli* in CLCSs, CAP treatment generated disinfectant properties in M-QW and NaCl solutions. Importantly, CAP-treated CLCSs significantly improved the cleaning performance on lipid-coated lenses, though M-QW’s cleaning ability worsened post-treatment. pH measurements indicated notable decreases in M-QW and NaCl after CAP, whereas buffered solutions like CLCSs and DPBS remained stable. Overall, CAP demonstrates promise for contact lens disinfection and surface modification; however, further research and pre-clinical trials are necessary before clinical application in ophthalmology.

Keywords: cold atmospheric plasma (CAP); plasma-activated solutions (PASs); reactive oxygen and nitrogen species (RONS); antibacterial effects; contact lens; contact lens cleaning solutions (CLCSs)



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1. Introduction

Cold atmospheric plasma (CAP) is an innovative technology in the medical field. It is a partially ionized gas near room temperature that generates reactive species that can kill bacteria, fungi, and viruses, making it useful for sterilization and disinfection [1–4]. CAP promotes wound healing by stimulating cell growth and reducing infection risks [5,6]. It is being examined for cancer therapy due to its ability to induce apoptosis in tumor cells [7]. Ongoing studies aim to expand its applications and ensure safety in medical use. CAP technology has also gained interest in the field of ophthalmology, and various research groups have extensively engaged with this topic in recent years.

Direct and indirect CAP treatments hold promise in ophthalmology for enhancing antimicrobial action, supporting wound healing, and modulating ocular surface physiology, with potential applications extending to contact lens care.

Direct CAP treatments involve applying plasma directly to the ocular surface or targeted tissues such as the corneal epithelium or conjunctiva. This approach can yield rapid antimicrobial effects and influence tissue remodeling, but it requires meticulous control of exposure time, distance, and gas composition to preserve the tear film, corneal endothelium, and intraocular structures.

Indirect CAP treatment pre-treats a solution (e.g., NaCl or water) with plasma, after which the generated plasma-activated solution (PAS) can be applied to the ocular surface and/or contact lenses. PASs can deliver reactive oxygen and nitrogen species (RONS) with potentially reduced direct tissue exposure and may allow more controllable dosing and deeper diffusion through the tear film. Given the short half-lives of many reactive species, detailed stability analyses are required to characterize decay kinetics and ensure consistent antimicrobial efficacy over the intended shelf life and usage period.

Many studies have already investigated the safety and effects of both direct and indirect CAP treatments in ophthalmology [8–12]. Histological examinations on ex vivo corneas and pig eyes after treating them with CAP were conducted; however, no histological changes were observed in the cornea, lens, or iris [13]. At the cellular level, a temporary increase in reactive oxygen species (ROS) was detected within conjunctival fibroblasts and keratocytes, which returned to baseline levels after 12–24 h [13].

In addition to reactive species, UVA and UVB radiation are generated during CAP production [14], and their harmful effects on the eye are already known [15]. These can manifest as photokeratitis, macular degeneration, or cataracts [16,17]. However, the use of indirect CAP treatment could help avoid this risk.

Other causes of keratitis include bacteria, viruses, fungi, and protozoa. The bacterial pathogens mainly involved are *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which can be eliminated with local broad-spectrum antibiotics [18,19]. Carbon dots (CDs) applications are also highlighted as promising ocular nanomedicine due to their antibacterial, antioxidant, anti-inflammatory, and anti-angiogenic properties, biocompatibility, tunable surface chemistry, and strong photoluminescence [20]. However, increasing resistance of bacteria to antibiotics makes successful treatment more difficult [21]. To date, no bacterial resistance has been observed with CAP treatment, which makes its use in keratitis therapy very promising [22]. Using a corneal stromal tissue model, a bactericidal effect against *Escherichia coli* (*E.coli*) was also demonstrated after a 5 min direct CAP treatment, effective up to a depth of 200 μm [23], indicating that CAP could also be suitable for the treatment of deep keratitis. Viruses such as adenoviruses and herpes simplex viruses (HSVs) can also cause keratitis. In corneal epithelial cells and corneas previously infected with HSV-1, treatment with CAP resulted in a dose-dependent reduction in the cytopathic effect, viral genome replication, and overall production of viral progeny [24]. Another form is acanthamoeba keratitis, which primarily occurs in contact lens wearers [25,26]. Acanthamoebae are pathogens that are ubiquitously present in the environment. They can exist either in the active form, the trophozoite stage, or, under life-threatening conditions, transition into the highly resistant dormant cyst stage [27]. In the cyst stage, Acanthamoebae are also resistant to disinfection and drying, making effective cleaning of contact lenses with multiple solutions impossible. As a result, they can remain viable and pathogenic for decades [28]. This is associated with partial resistance to currently available medications, making treatment with CAP an interesting option. Surprisingly, it has been found that CAP can inactivate Acanthamoebae in both the trophozoite and cyst stages, and this ability is

also exhibited on contact lens materials [27]. Additionally, CAP-treated hydrogels placed on infected donor corneas enabled potent inactivation of *Acanthamoebae* [28].

Another application of CAP involves treating the contact lens itself. Good contact lens hygiene is a fundamental prerequisite for the prevention of contact lens-related complications. According to Roth et al. (2002), 60% of infections in contact lens wearers are associated with inadequate hygiene conditions during lens care [29]. The purpose of contact lens care is to protect the eye from infections, maintain the lens's performance throughout the prescribed wearing period, and ensure maximum comfort during wear [30]. It consists of several components, including cleaning, disinfection, storage, and wetting of the lens [31].

CAP treatment (either directly or indirectly) can modify the surface properties of lenses to reduce microbial adhesion and effectively remove manufacturing residues that may cause discomfort or infections [32]. Additionally, during this treatment, the wettability angle of the contact lens is reduced, thereby improving the lens's wettability and overall comfort in the eye [33]. Besides increased wettability, a reduction in bacterial adhesion [34], a decrease in protein and cell deposits, and an increase in oxygen permeability have also been observed [35]. Furthermore, the sterilizing effect of CAP on contact lenses is of great significance [36].

However, there are different CAP types that could potentially be used on contact lenses (both rigid and flexible) for surface modification or functionalization, e.g., Dielectric Barrier Discharge (DBD) Plasma, Surface Micro Discharge (SMD) Plasma, Atmospheric Pressure Plasma Jet (APPJ), or Plasma Torch systems. Comparative studies are needed to determine which types of plasma devices are best for cleaning and sterilizing lenses. However, the selection strongly depends on lens material (PMMA, hydrogel, silicone hydrogel) and intended functionalization (antimicrobial, wettability, protein resistance).

Numerous studies report positive effects of both direct and indirect CAP treatments in ophthalmology, yet many questions remain unresolved. Further research is needed to optimize the production of PASs, evaluate long-term safety, and improve their cleaning performance. Thus, the present study focuses on indirect CAP treatments, specifically on the generation, stability, and cleaning efficacy of various PASs.

2. Materials and Methods

2.1. Cold Atmospheric Plasma Device

A prototype of the plasma care[®] device developed by terraplasma GmbH, Garching, Germany, was used for CAP treatment. A recent publication detailed the design, technology, and ozone emission spectrum of this device [37]. The device employs a technology known as "thin-film technology", which is an advanced version of surface micro-discharge technology (SMD) [38]. The application of a high voltage of 3.5 kV generates millimeter-sized micro-discharges within the plasma-source unit. The unit consists of a high-voltage electrode, a dielectric, and a grounded structured electrode [37], which together produce plasma components that can be adjusted based on frequency and voltage.

Although the device is primarily designed for surface treatments, previous studies using the plasma care[®] device in a "closed system" (using a spacer) demonstrated its effectiveness in the production of large volumes of PASs [37,39].

2.2. Treatment of Different Solutions with Cold Atmospheric Plasma

Various solutions were treated with CAP. In addition to Milli-Q water (M-QW; Enviro-FALK GmbH, Prozesswasser-Technik, Industriegebiet Sainscheid, Gutenbergstraße 7, 56457 Westerburg, Germany), physiological saline (0.9% NaCl; B. Braun Melsungen AG, Melsungen, Germany), Dulbecco's Phosphate Buffered Saline (DPBS; Fisher Scientific GmbH,

Schwerte, Germany), and three different contact lens cleaning solutions with varying compositions were used. These included the Biotrue “All-in-One” solution (BT; Bausch + Lomb, Rochester, NY, USA), the Boston[®] One-Bottle Conditioning Solution (B&L; Bausch + Lomb, Rochester, NY, USA), and the Eyemax24 contact lens solution (EM; MPG&E Handel und Service GmbH, Bordesholm, Germany). A total of 2.5 mL of each of the solutions mentioned above was transferred into a 35 mm cell culture dish (Primaria[™] Easy Grip Cell Culture Dish, Corning Incorporated, New York, NY, USA). The filled culture dish, together with a magnetic stir bar, was placed on a magnetic stirrer, which had been pre-set to 150–200 revolutions per minute (rpm).

For all experiments, the plasma device was operated at a frequency of 4 kHz. Depending on the experimental setup, the dose of CAP varied between 1, 2, 5, and 10 min. The plasma source, including a spacer, was positioned above the culture dish, and the CAP treatment was initiated. Since the diameter of the culture dish matched that of the spacer, placing the spacer on the dish created a closed system, preventing the escape of plasma components.

2.3. Determination of Reactive Species in Short- and Long-Term Experiments

Following CAP treatment, the generated reactive species in the solutions were analyzed using different methods and compared with the respective untreated controls. The focus was placed on dihydrorhodamine 123 (DHR123) as an indicator of the presence of reactive oxygen species (ROS), hydrogen peroxide (H₂O₂), nitrite (NO₂[−]), and nitrate (NO₃[−]). Depending on the experimental setup, measurements were carried out either immediately after CAP treatment (short-term experiment) or one to four weeks later (long-term experiment). For the long-term experiment, both untreated and treated solutions were transferred into 25 mL screw-cap vials (SARSTEDT AG & CO. KG, Nümbrecht, Germany) and stored in the dark at room temperature until measurement. For quantification of H₂O₂, a Fluorimetric Hydrogen Peroxide Assay Kit (Sigma Aldrich GmbH, Steinheim, Germany) was used, and the fluorescence was measured at an excitation wavelength of 540 nm and an emission wavelength of 590 nm. NO₂[−] and NO₃[−] concentrations were determined using the colorimetric Nitrite/Nitrate Assay Kit (Sigma Aldrich GmbH, Steinheim, Germany) to detect nitric oxide metabolites at 540 nm absorbance. Dihydrorhodamine 123, 10 μM (DHR123; Sigma-Aldrich GmbH, Steinheim, Germany) was dissolved in 100 μL of the solution of interest. The solution was then transferred to a black 96-well plate (Greiner Bio-One GmbH, Frickenhausen, Germany), and fluorescence was recorded with a plate reader (Varioscan Flash, Thermo Fisher, Schwerte, Germany) at an excitation wavelength of 505 nm and an emission wavelength of 534 nm. All determinations of reactive species in both short- and long-term experiments were conducted $n = 3$ in duplicates, and the results were averaged.

2.4. Antibacterial Effects of Various Plasma-Treated Solutions on *Escherichia coli*

A total of 400 μL of bacteria suspension (~10⁶ /mL; *E. coli* ATCC 25922; Thermo Scientific[™], Bishop Meadow Road, Loughborough, Leicestershire, LE11 5RG) was transferred into seven 1.5 mL Eppendorf reaction tubes and centrifuged again at 5.000 rpm for 5 min. The supernatant was discarded, and the resulting bacterial pellet was resuspended in 400 μL of one of the following solutions: M-QW, NaCl, CLCS (BT, EM, B&L), and 3% H₂O₂ (Sigma-Aldrich, St. Louis, MO, USA). M-QW was used untreated as a negative control, while M-QW treated with CAP for 10 min served as the experimental variant. In contrast, 3% H₂O₂ was applied as positive control.

After an exposure time of one hour, 200 μL from each Eppendorf tube was taken and transferred to the first column of a 96-well plate (Corning Incorporated, New York,

NY, USA). Experiments with extended exposure times of the different solutions for *E. coli* were also performed. Here, the exposure time of the solutions on the bacterial culture was extended from one hour to 4 or 24 h. The dilution series in the 96-well plate was therefore prepared only 4 or 24 h after the solutions had been added to the 1.5 mL Eppendorf reaction tubes containing the bacterial culture. The subsequent experimental procedure and selection of solutions was performed identically. A serial dilution was then prepared in DPBS (Fisher Scientific GmbH, Schwerte, Germany) at a ratio of 1:10. The different dilutions of each solution were subsequently plated onto a Mueller–Hinton agar plate (Institute of Microbiology and Hygiene, University of Regensburg, Regensburg, Germany), which had been divided into seven equal sections. For each section, three 20 μ L drops of the same dilution (ranging from 10^{-2} to 10^{-8}) were applied based on the method published by Miles et al. (1938) [40]. This step was carried out on a sterile workbench. Once the drops had dried, the Mueller–Hinton plates were incubated overnight at 37 °C. The following day, the colony forming units (CFUs) were evaluated. Each experiment was carried out $n = 3$, and the results were averaged.

2.5. Cleaning of Lipid-Coated Silicone Hydrogel Contact Lenses with Different Plasma-Treated and Untreated Contact Lens Solutions

The contact lenses (silicone hydrogel daily disposable contact lenses; ACUVUE® oasys with HydraLuxe™, Johnson & Johnson Medical GmbH, Jacksonville, FL, USA) were stored in olive oil (Chiavalon; 52215 Vodnjan, Istria, Croatia) at room temperature for 24 h before being cut in half with scissors. As the establishment of a homogeneous cholesterol-based lipid coating on the contact lenses proved challenging during the preliminary experiments, native olive oil was employed in the experiments described herein, as it yielded a relatively uniform distribution of lipid droplets. This uniformity facilitates reproducible interaction with lens surfaces and PAs, reducing variability attributable to lipid inhomogeneity. While olive oil is not identical to human meibomian secretions, its lipid-rich composition and capacity to form a more homogeneous coating make it a reasonable surrogate for evaluating the handling, cleaning efficacy, and lipid-related interactions under study conditions. We point out that olive oil is a model system and we justify its use as a practical, consistent proxy to enable controlled comparisons and interpretable results, with the caveat that extrapolation to human native secretions should be made cautiously.

Subsequently, the oil-coated lens halves were placed in different cleaning solutions for 24 h. The cleaning solutions used were M-QW, BT, EM, B&L, as well as 3% H₂O₂. These solutions were either pretreated with CAP for 10 min or applied untreated. H₂O₂, 3% served as the positive control in this experiment and was not pretreated with CAP. After preparation of the Nile Red–glycerol solution (Sigma-Aldrich, St. Louis, MO, USA), as previously described by Pucker and Nichols (2012) [41], the lens halves were removed from the different cleaning solutions and placed on a glass slide (Fisher Scientific, Pittsburgh, USA). Subsequently, 100 μ L of the Nile Red–glycerol solution was applied to each lens for 10 min, during which the samples were kept in the dark. After this incubation period, the Nile Red–glycerol solution was rinsed off the lens surface with 1 mL DPBS (Fisher Scientific GmbH, Schwerte, Germany). To prevent dehydration, the lenses were immediately moistened with 100 μ L of DPBS on the slide. For fluorescence microscopy, the pretreated contact lenses were transferred to a fresh glass slide, and excess DPBS was carefully removed with a paper towel. For each lens half, two images from different areas were acquired at 5 \times magnification using the Axio Imager Z1 (Carl Zeiss Vision, Hallbergmoos, Germany). The most distinct fluorescence signal was obtained at an excitation wavelength range of 542–554 nm. For data analysis, all lipid droplets per image were counted, and the mean value as well as the standard deviation from the two recordings were determined. Plasma-treated solutions were compared with the corresponding untreated solutions, and

the percentage of lipid droplets remaining on the lens surface was calculated. The number of lipid droplets observed in the untreated solutions was defined as 100%. Statistical significance was determined in reference to the respective untreated solution. The experiment was conducted $n = 4$ in duplicates.

2.6. pH Measurement of Plasma-Treated Solutions

The pH values of untreated and CAP-treated CLCS, M-QW, NaCl, and DPBS were determined. CAP exposure times of 1, 2, and 5 min were applied. Immediately after treatment, the solutions were transferred into 5 mL Eppendorf reaction tubes, and the pH was measured using a pH meter (Mettler-Toledo GmbH, Gießen, Germany). Each measurement was performed $n = 3$ in duplicates. From the obtained pH-values, the mean and standard deviation were calculated. Statistical evaluation was performed by two-way ANOVA with Dunnett's multiple comparisons test. Statistical significance was determined in reference to the respective untreated solution.

2.7. Statistical Analysis

All data were analyzed with GraphPad Prism software (GraphPad Software Inc., San Diego, CA, USA) version 10.4.1. and expressed as mean \pm standard deviation (SD). Two-way ANOVA with Dunnett's multiple comparisons test was done to indicate differences of the mean within the untreated control and the CAP-treatment groups. An unpaired t test or Mann-Whitney test was used to compare the control group within the corresponding CAP treated group as indicated in the corresponding figure legend. Significant results are indicated * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, or **** $p < 0.0001$; ns (not significant).

2.8. Objectives and Flow Chart of the Experiments

In the past, CAP has been shown to possess good antibacterial properties, which can also be transferred to various media [42,43].

This observation ultimately shaped the objectives of the present study. Importantly, this study does not focus on direct plasma treatment of contact lenses for cleaning and sterilization. Instead, it investigates the application of different PASs and their effects on the lenses. The study examines whether CAP treatment of CLCS and other liquids, such as M-QW, NaCl, and DPBS, could enhance contact lens cleaning. Functional changes were analyzed by measuring RONS generated immediately after CAP treatment, and RONS stability was monitored long-term and evaluated over a period of up to four weeks. Furthermore, the antibacterial effect of the CAP-treated solutions on *E. coli* was examined as a function of exposure time. This study also assessed the impact of CAP treatment on the cleaning efficiency of different solutions against lipid-coated contact lenses, before finally determining the extent to which CAP treatment influenced the pH value of the tested solutions (Figure 1).

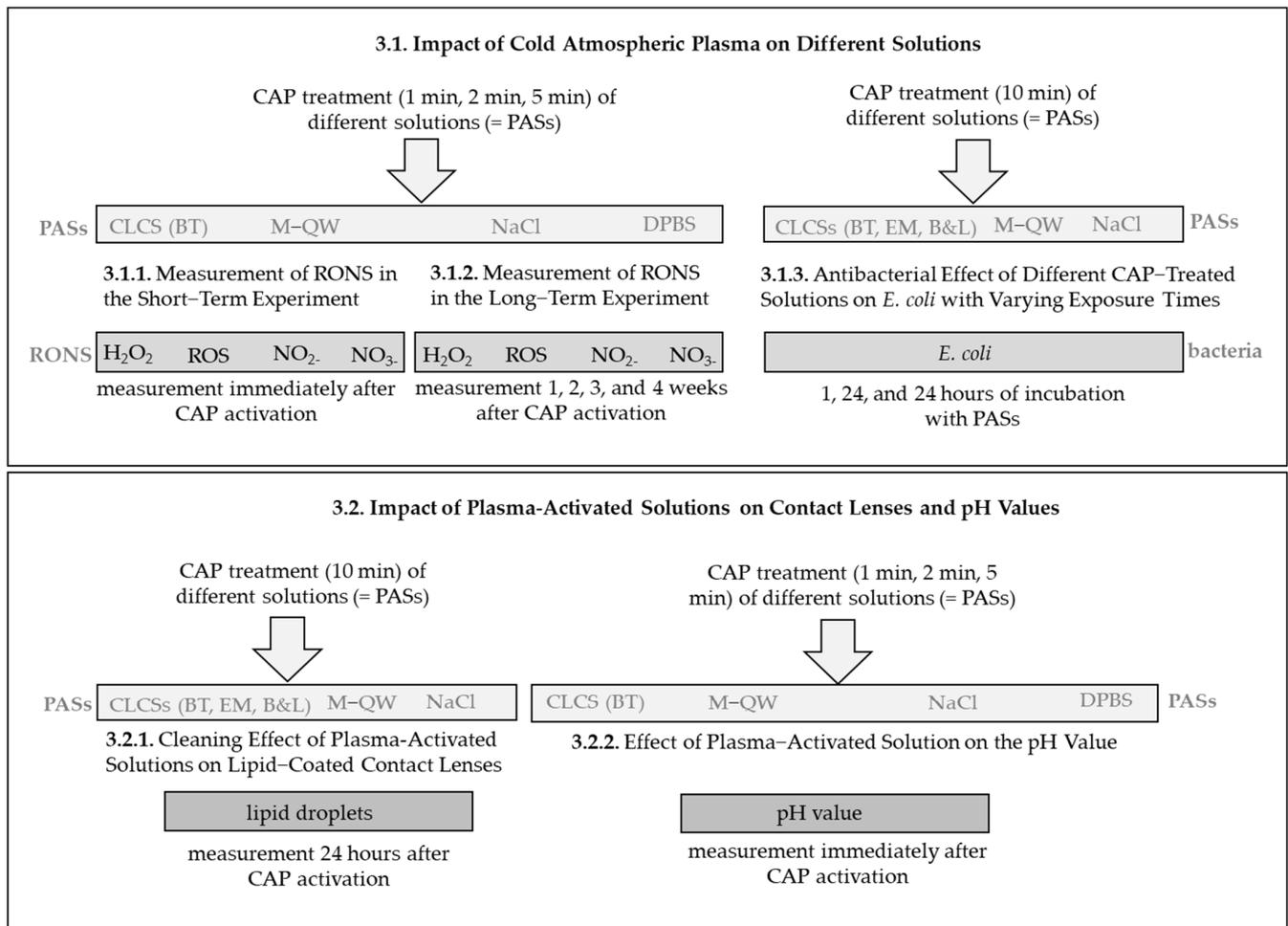


Figure 1. Flow chart of the experiments. The study begins with CAP treatment (1 min, 2 min, 5 min) of different solutions (plasma-activated solutions, PASs): contact lens cleaning solution (CLCS) from Biotrue (BT) and other liquids (M-QW, NaCl, DPBS). Immediately after CAP treatment, functional changes are assessed by measuring reactive oxygen and nitrogen species (RONS): H₂O₂, ROS, NO₂⁻, and NO₃⁻ (Section 3.1.1). The next step is monitoring the stability of RONS over a long-term period of up to four weeks (Section 3.1.2). Concurrently, the antibacterial effect of PASs (CLCSs from Biotrue (BT), Eyemax24 (EM), Bausch + Lomb (B&L), M-QW, and NaCl) on *E. coli* is examined as a function of exposure time (Section 3.1.3). This study also evaluates the cleaning efficiency of the different PASs against lipid-coated contact lenses (Section 3.2.1). Finally, this research determines how CAP treatment influences the pH value of the tested solutions (Section 3.2.2).

3. Results

3.1. Impact of Cold Atmospheric Plasma on Different Solutions

3.1.1. Measurement of Reactive Oxygen and Nitrogen Species in the Short-Term Experiment

In the short-term experiment, the intensity of RONS was measured in four different solutions immediately after treatment with CAP. The aim was to assess the direct impact of CAP on RONS levels compared to untreated solutions. The tested solutions were a commercial CLCS (BT; Biotrue “All-in-One” by Bausch + Lomb), M-QW, NaCl, and DPBS. In the short-term experiment, this study also aimed to determine whether there is a correlation between RONS intensity and the CAP treatment dose. The four solutions were treated with CAP for 1, 2, and 5 min. In total, the intensities of four different RONS were measured: hydrogen peroxide (H₂O₂) (Figure 2a) and other reactive oxygen species (ROS) using a DRH 123 assay kit (Figure 2b), as well as the reactive nitrogen species nitrite (NO₂⁻) (Figure 2c) and nitrate (NO₃⁻) (Figure 2d). All CAP-treated solutions showed an

increase in H_2O_2 , ROS, NO_2^- , and NO_3^- in comparison to the untreated control. The most pronounced increases were observed for H_2O_2 (~600-fold induction in CLCS after 5 min CAP treatment), for NO_2^- (~250-fold induction in DPBS after 5 min CAP treatment) and NO_3^- (~4.000-fold induction in M-QW after 5 min CAP treatment), while the rise in ROS was generally small across all solutions (~5-fold induction in M-QW after 1 min CAP treatment). Moreover, the intensity of reactive species was, in most cases, dependent on the CAP dose.

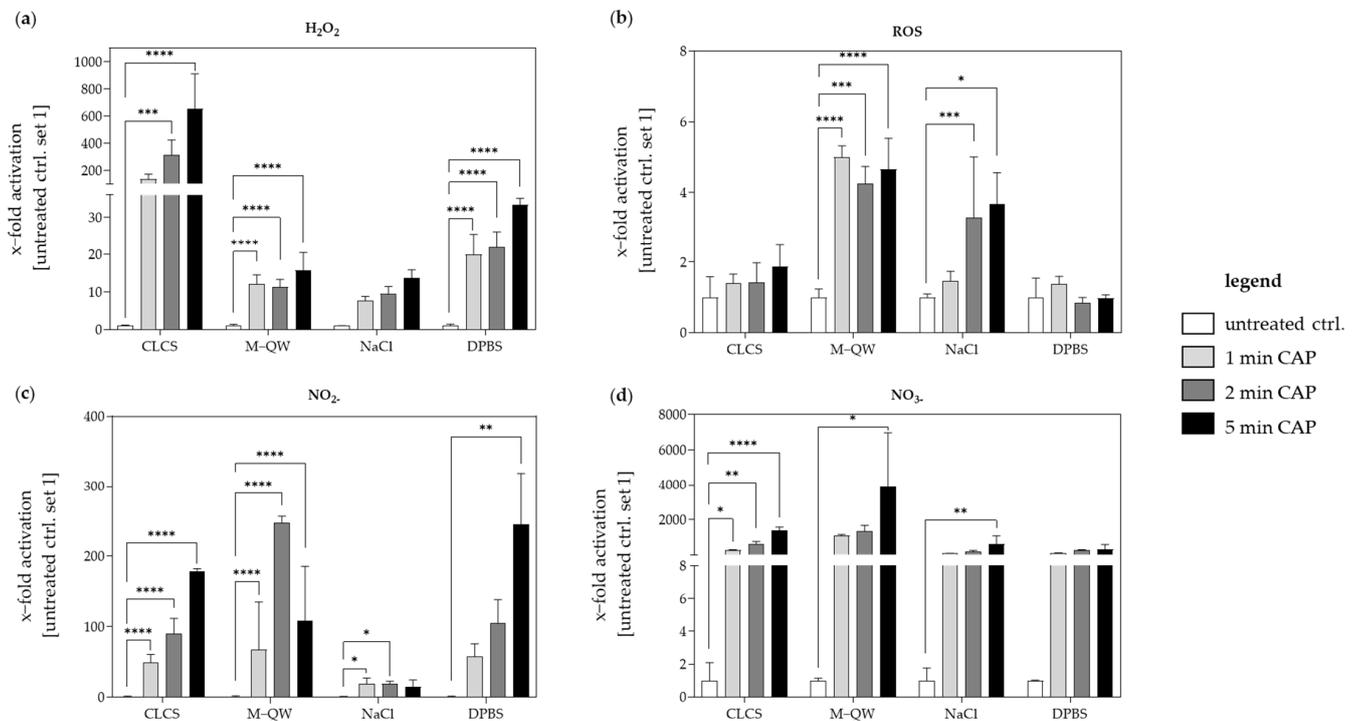


Figure 2. RONS measurement in the short-term experiment. (a) H_2O_2 , (b) ROS (DRH 123), (c) nitrite (NO_2^-), and (d) nitrate (NO_3^-) intensity was measured in CLCS (BT), M-QW, NaCl, and DPBS in the untreated state and after 1-, 2-, and 5-min CAP treatment. The untreated control was set to 1. Statistical significance refers to comparisons between untreated and CAP-treated samples. Analysis was performed using two-way ANOVA with Dunnett's multiple comparisons test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

3.1.2. Measurement of Reactive Oxygen and Nitrogen Species in the Long-Term Experiment

In the short-term experiment, the RONS intensity was measured immediately after CAP treatment in different solutions. Since most CLCSs can be used up to 90 days after opening, this study investigated whether RONS remain detectable weeks after treatment. The same solutions from Section 3.1.1. were treated with CAP for 1, 2, and 5 min, then incubated in the dark at room temperature in screw-cap tubes for up to 4 weeks. After 1, 2, 3, and 4 weeks, the intensities of the RONS were measured and compared with those of untreated solutions.

Measurement of ROS (DHR123)

In the four-week experiments, CAP treatment again led to slight but distinct changes in ROS intensity depending on the solution. In CLCSs, a dose-dependent increase in ROS was observed compared to the untreated controls; however, the overall ROS levels declined slightly over the four-week period compared to the short-term (immediate) results (Figure 3a). In M-QW, a ~4–5-fold increase in ROS was detected immediately after CAP treatment compared to the control. This effect decreases in weeks 3 and 4, returning almost

to the values of the untreated control (Figure 3b). Just like in the short-term experiment, a slight increase in ROS was observed in all treated NaCl samples after four weeks compared to the untreated solution. However, in comparison to the ROS intensity measured in the short-term experiment, these values declined slightly over the four-week period (Figure 3c). In DPBS, CAP treatment caused a slight decrease in ROS relative to the untreated sample, with an additional reduction during the four weeks (Figure 3d). The overall observed decline in ROS intensity over four weeks likely reflects the rapid decay of short-lived radical species generated by CAP and their conversion into longer-lived, less reactive products, e.g., H_2O_2 .

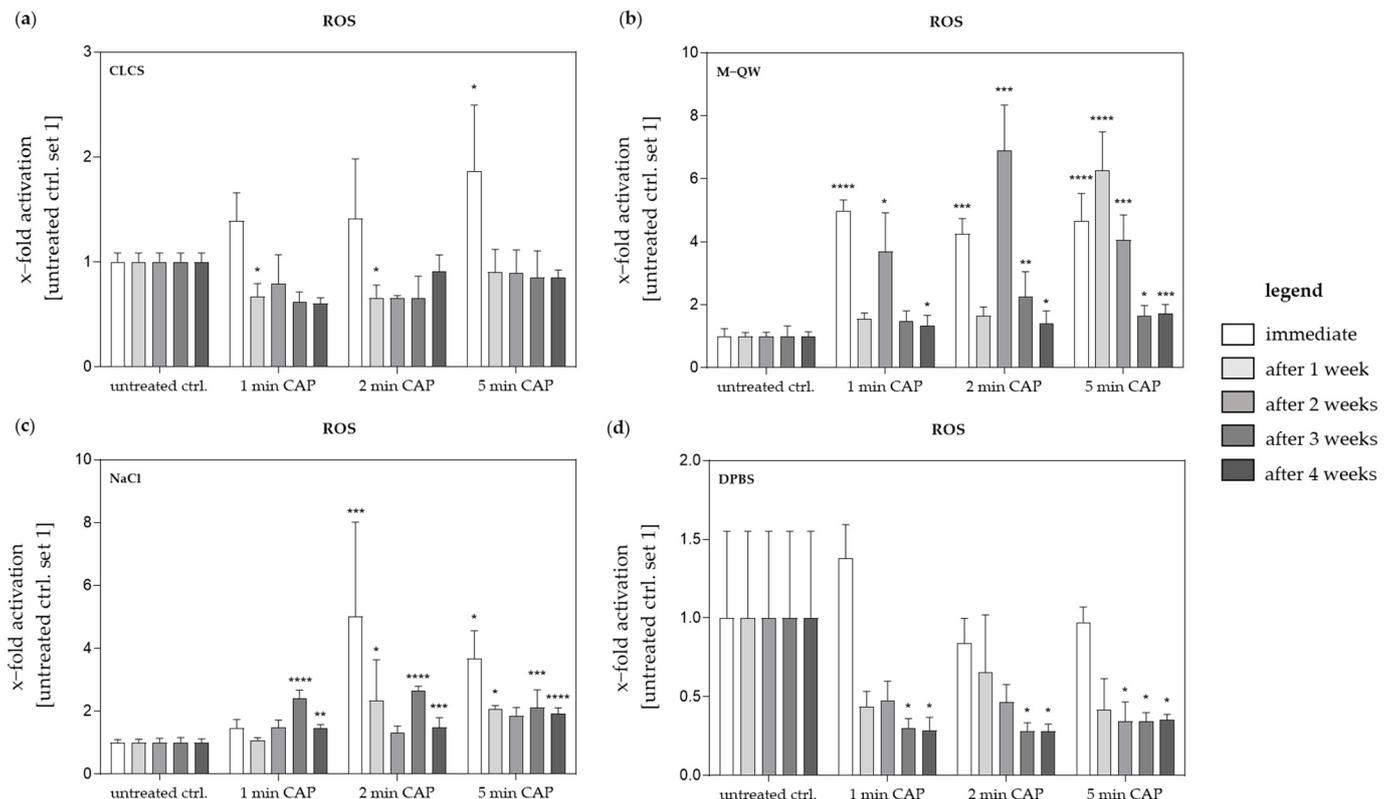


Figure 3. ROS measurement in the long-term experiment. ROS intensity was measured in (a) CLCS (BL), (b) M-QW, (c) NaCl, and (d) DPBS in the untreated state and after 1-, 2-, and 5-min CAP treatment immediately after CAP treatment, as already shown in Figure 1, and 1–4 weeks later. The untreated control was set to 1. Statistical significance refers to comparisons between untreated and CAP-treated samples. Analysis was performed using two-way ANOVA with Dunnett's multiple comparisons test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Measurement of H_2O_2

In the long-term experiment, CAP treatment of CLCS resulted in a strong dose-dependent increase in H_2O_2 (~300–600-fold) compared to the untreated control; however, these levels declined again over the four-week period relative to the short-term (immediate) results (Figure 4a). In M-QW, there was a maximum 10–15-fold increase in H_2O_2 , which remained relatively stable over the 4 weeks and showed no CAP dose dependence (Figure 4b). In NaCl, a slight reduction over time could be observed compared to the values measured immediately after treatment. Interestingly, the high values after 4 weeks (e.g., 17-fold induction after 2 min CAP) suggest a boost and long-term stability of H_2O_2 in this solution (Figure 4c). In DPBS, CAP treatment caused a dose-independent increase in H_2O_2 , which remained fairly stable throughout the four weeks (Figure 4d). The solution-dependent

differences in H_2O_2 formation and stability after CAP reflect the interplay of initial H_2O_2 generation yield and matrix chemistry (organics, salts, buffers).

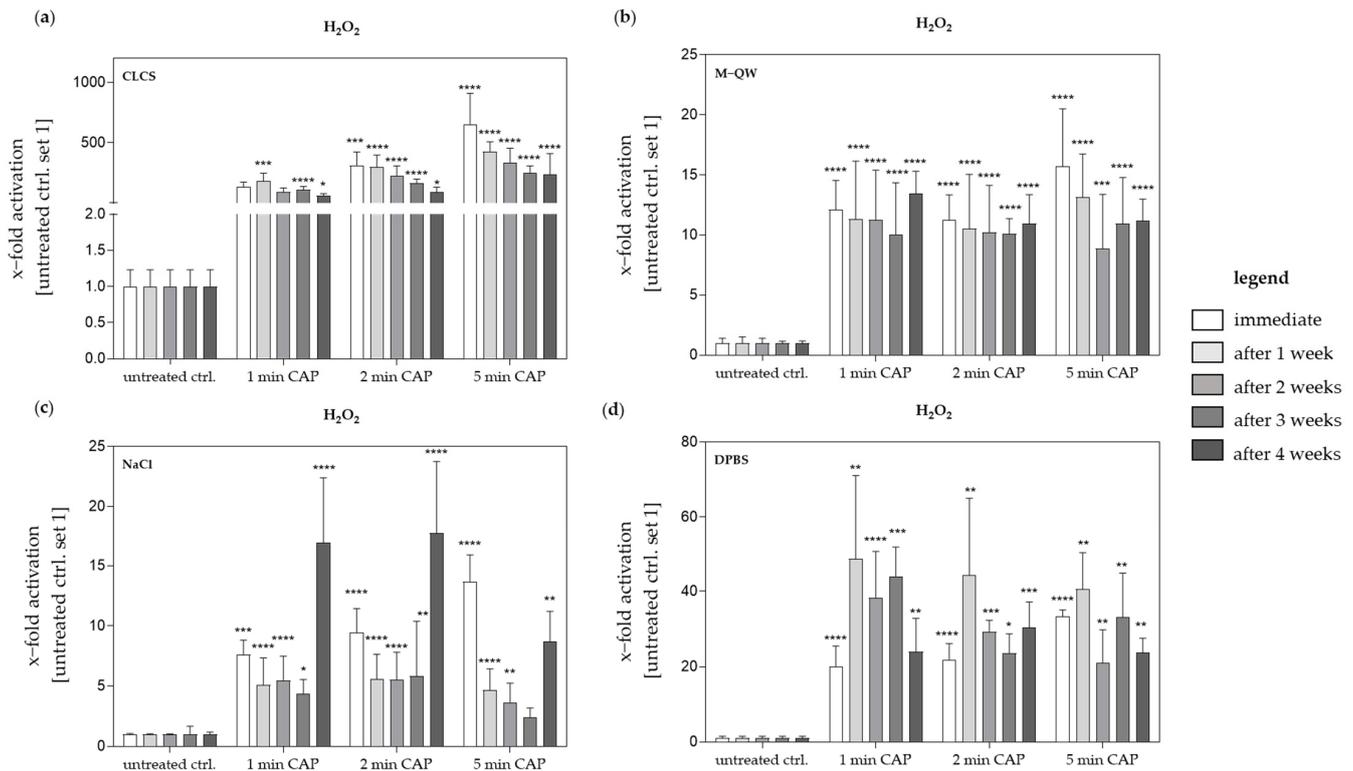


Figure 4. H_2O_2 measurement in the long-term experiment. H_2O_2 intensity was measured in (a) CLCS (BL), (b) M-QW, (c) NaCl, and (d) DPBS in the untreated state and after 1-, 2-, and 5-min CAP treatment immediately after CAP treatment, as already shown in Figure 1 and 1–4 weeks later. The untreated control was set to 1. Statistical significance refers to comparisons between untreated and CAP-treated samples. Analysis was performed using two-way ANOVA with Dunnett's multiple comparisons test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Measurement of Nitrite (NO_2^-)

As in the short-term trial, CAP treatment led to a strong dose-dependent increase in NO_2^- in CLCS, remaining constant over the four-week period (Figure 5a). In M-QW, all CAP-treated samples showed a dose-independent increase in NO_2^- after one week (~10–80-fold) compared to untreated ones. However, this effect was reduced to baseline levels after two, three and four weeks (Figure 5b). In all long-term CAP-treated NaCl samples, a slight dose-independent increase in NO_2^- was observed with highest induction after one week, which tended to decrease over four weeks (Figure 5c). Long-term CAP treatment also caused a strong dose-dependent increase in NO_2^- in DPBS, which stayed stable over four weeks (Figure 5d). It can therefore be concluded that the long-term stability of NO_2^- in CAP-treated solutions mainly depends on buffering capacity. Buffered solutions like DPBS and CLCS maintain nitrite, whereas unbuffered ones like M-QW and NaCl allow its conversion or degradation over time.

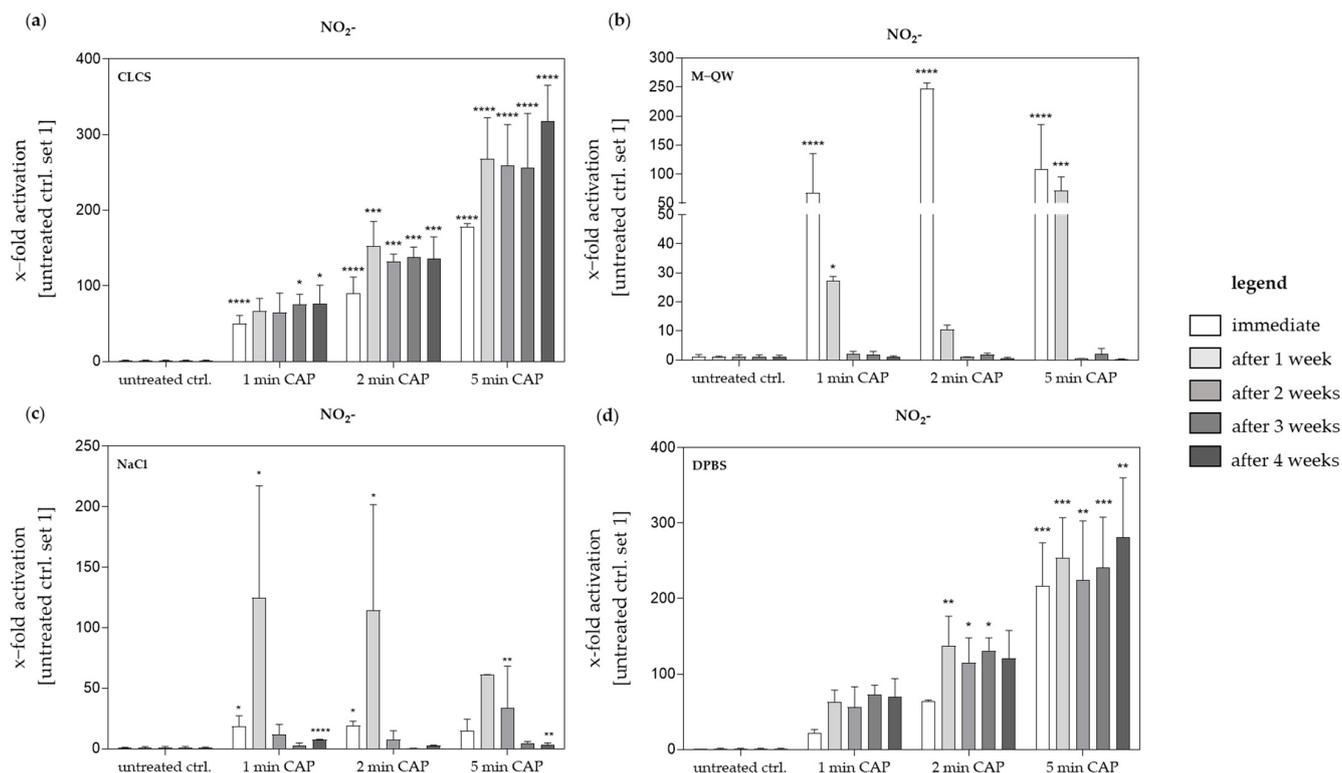


Figure 5. NO_2^- measurement in the long-term experiment. NO_2^- intensity was measured in (a) CLCS (BL), (b) M-QW, (c) NaCl, and (d) DPBS in the untreated state and after 1-, 2-, and 5-min CAP treatment immediately after CAP treatment, as already shown in Figure 1, and 1–4 weeks later. The untreated control was set to 1. Statistical significance refers to comparisons between untreated and CAP-treated samples. Analysis was performed using two-way ANOVA with Dunnett’s multiple comparisons test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Measurement of Nitrate (NO_3^-)

A similarly very strong dose-dependent rise in NO_3^- was also seen in all long-term CAP-treated CLCS samples (~1.000–5.000-fold), which remained stable throughout the four weeks (Figure 6a). In M-QW, all long-term CAP-treated samples showed a very strong dose-independent increase in NO_3^- compared to untreated ones. However, compared to the short-term trial, NO_3^- levels decreased slightly over the four weeks (Figure 6b). In all long-term CAP-treated NaCl samples, a very strong dose-dependent increase in NO_3^- was observed (~1.000–2.000-fold) compared to untreated samples, remaining fairly stable over the four weeks (Figure 6c). In DPBS, a dose-independent increase in NO_3^- was observed (~100–500-fold) in all CAP-treated samples, staying very stable over the four-week period (Figure 6d).

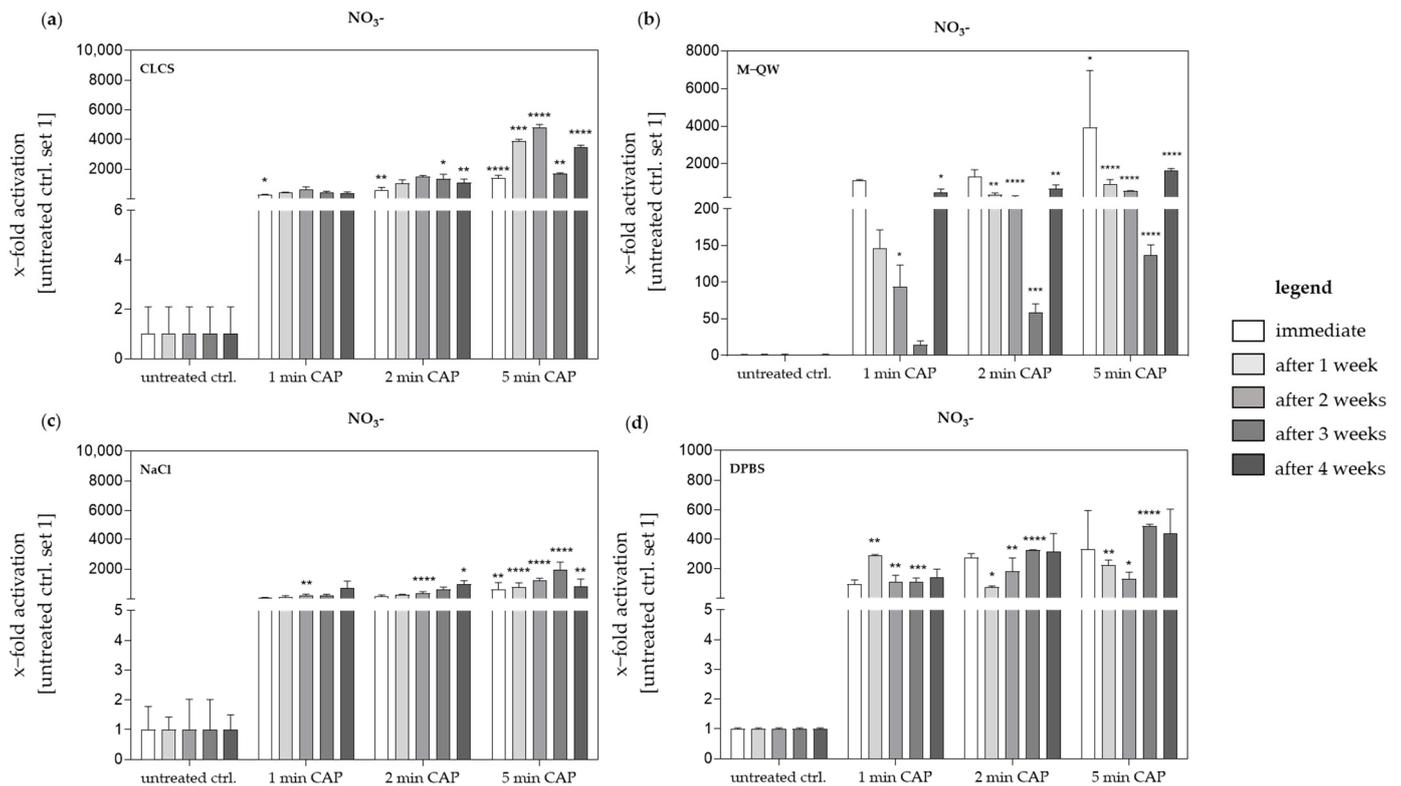


Figure 6. NO_3^- measurement in the long-term experiment. NO_3^- intensity was measured in (a) CLCS (BL), (b) M-QW, (c) NaCl, and (d) DPBS in the untreated state and after 1-, 2-, and 5-min CAP treatment immediately after CAP treatment, as already shown in Figure 1, and 1–4 weeks later. The untreated control was set to 1. Statistical significance refers to comparisons between untreated and CAP-treated samples. Analysis was performed using two-way ANOVA with Dunnett’s multiple comparisons test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

3.1.3. Antibacterial Effect of Different CAP-Treated Solutions on *Escherichia coli* with Varying Exposure Times

The antibacterial effect of many “All-in-One” solutions is considered inadequate. For the functional antibacterial analyses, we conducted dose–response CAP treatments at 2, 5, and 10 min (Figure 7a) and found that the antibacterial effect was most pronounced at 10 min using M-QW for CAP activation. Consequently, all subsequent bacterial assays (Figure 7b–d) were performed with a 10-min CAP treatment, because a 10-min exposure provides a consistent and robust condition to evaluate functional outcomes. Next, M-QW, NaCl and different CLCSs were tested to determine whether the antibacterial effect could be improved by CAP. In addition to the BT CLCS, the Eyemax24 CLCS from MPG&E Handel and Service GmbH (EM) and the Boston® one-bottle combination solution from Bausch + Lomb (B&L) were added. Furthermore, the question arose whether prolonging the exposure time of the contact lens solutions could improve their antibacterial effect. Therefore, in this experiment, the exposure time was extended from 1 h (Figure 7b) to 4 h (Figure 7c) and 24 h (Figure 7d). In this way, it was to be determined whether the antibacterial effect of the untreated and 10 min CAP-treated solutions depends on the exposure time. A 3% H_2O_2 solution was used as the positive control in this experiment. In M-QW and NaCl, a 10 min CAP treatment resulted in an improved antibacterial effect against *E. coli* compared to the untreated solutions (Figure 7b). The CAP-treated M-QW reduced CFUs by more than 99.999%. For the three tested CLCSs, the antibacterial effect remained relatively unchanged after the 10 min CAP treatment. In the H_2O_2 positive control, CFU numbers were below the detection limit. After four hours of exposure, the CAP-treated M-QW and NaCl solution again showed a stronger antibacterial effect

compared to their respective untreated samples. The effect was more pronounced than after one hour, with CFU counts for both solutions falling below the detection limit (Figure 7c). The antibacterial activity of the three untreated CLCSs after four hours was similar to the results described after one hour of incubation. Likewise, CAP treatment of the CLCSs did not lead to any notable improvement in antibacterial activity. As before, CFU numbers in the positive control were below the detection limit. After 24 h (Figure 7d), the antibacterial effect of the CAP-treated M-QW and NaCl was comparable to the results from Figure 7c, with CFU counts remaining below the detection limit. Among the three untreated CLCSs, a generally improved antibacterial effect against *E. coli* was observed after the extended exposure time. The antibacterial effect of BT remained <99.9% reduction, but CFU counts declined by 1.5 log₁₀ units (from ~ 5.5 to 4) with elevated CAP exposure time from 4 h (Figure 7c) to 24 h (Figure 7d). For EM, the antibacterial effect increased to 99.999% reduction (Figure 7d). B&L showed the strongest reduction, with CFU counts dropping below the detection limit after 24 h (Figure 7d). In contrast, the CAP-treated BT and EM solutions showed a weaker reduction in CFUs compared to their respective untreated samples, while no difference was observed between treated and untreated B&L. In summary, treatment of M-QW and NaCl with CAP resulted in a strong antibacterial effect against *E. coli*. Even after just one hour of exposure, a clear reduction in CFU was observed for both pre-treated solutions. After four hours, the antibacterial effect of CAP-treated M-QW and NaCl was comparable to that of H₂O₂. In contrast, the antimicrobial properties of the three selected CLCSs were only slightly affected by CAP treatment.

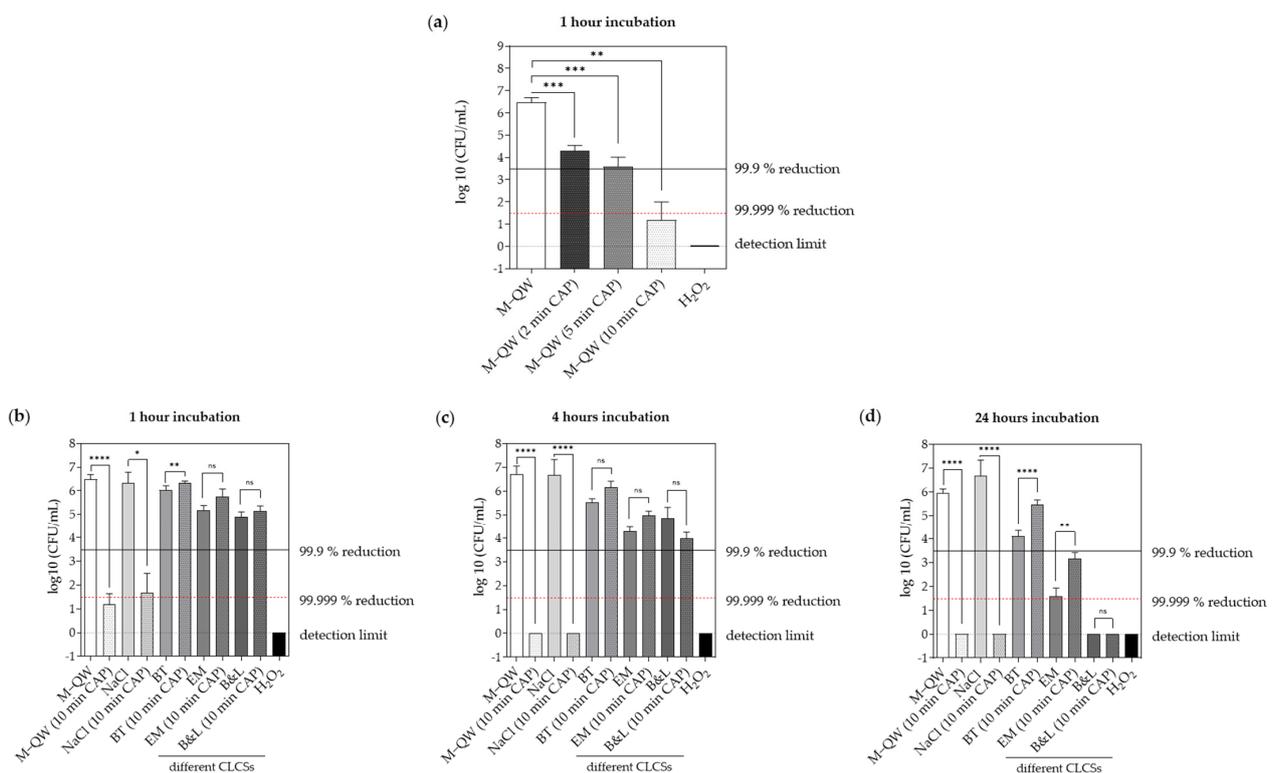


Figure 7. (a) Antibacterial effect of CAP-treated M-QW on *E. coli* and antibacterial effect of different solutions after (b) 1 h, (c) 4 h, and (d) 24 h of incubation. The log₁₀ colony forming units (CFU)/mL in (b–d) are shown after ten minutes of CAP treatment and in the corresponding untreated solution. 10 min exposure provides a consistent and robust condition to evaluate functional outcomes, as shown in (a). H₂O₂ serves as the positive control. Statistical significance refers to the untreated solution. Statistical analysis: Mann–Whitney test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; ns (not significant).

3.2. Impact of Plasma-Activated Solutions on Contact Lenses and pH Values

3.2.1. Cleaning Effect of Plasma-Activated Solutions on Lipid-Coated Contact Lenses

In addition to contamination with bacteria, lipid deposits also form on the lens during contact lens use. In addition to physiological lipids from the tear film, lipids from creams or cosmetics can also get onto the lens surface. These deposits in turn promote the adhesion of bacteria, which is why cleaning the surface of lipid components with contact lens solutions plays an essential role in addition to their antibacterial effect. For this, it was analyzed whether a 10 min CAP treatment has a positive effect on the lipid cleaning effect of M-QW and different CLCSs. In addition to the BT, the Eyemax24 (EM) and the Boston® one-bottle combination solution from Bausch + Lomb (B&L) were added as already used in Section 3.1.3. A 10 min CAP treatment significantly improved the cleaning efficiency of the BT “All-in-One” solution on lipid-coated contact lenses compared to the untreated solution. After cleaning with the CAP-treated BT solution, 73% fewer lipid droplets were detected on the lens surface, as shown in Figure 8a and exemplified in Figure 8b. The other two CLCSs (EM and B&L) also showed a trend toward improved lipid removal after CAP treatment, with reductions of 27.6% and 47.4%, respectively. In contrast, CAP treatment did not enhance the cleaning performance of M-QW, while 3% H₂O₂ was used as the positive control and showed good treatment results.

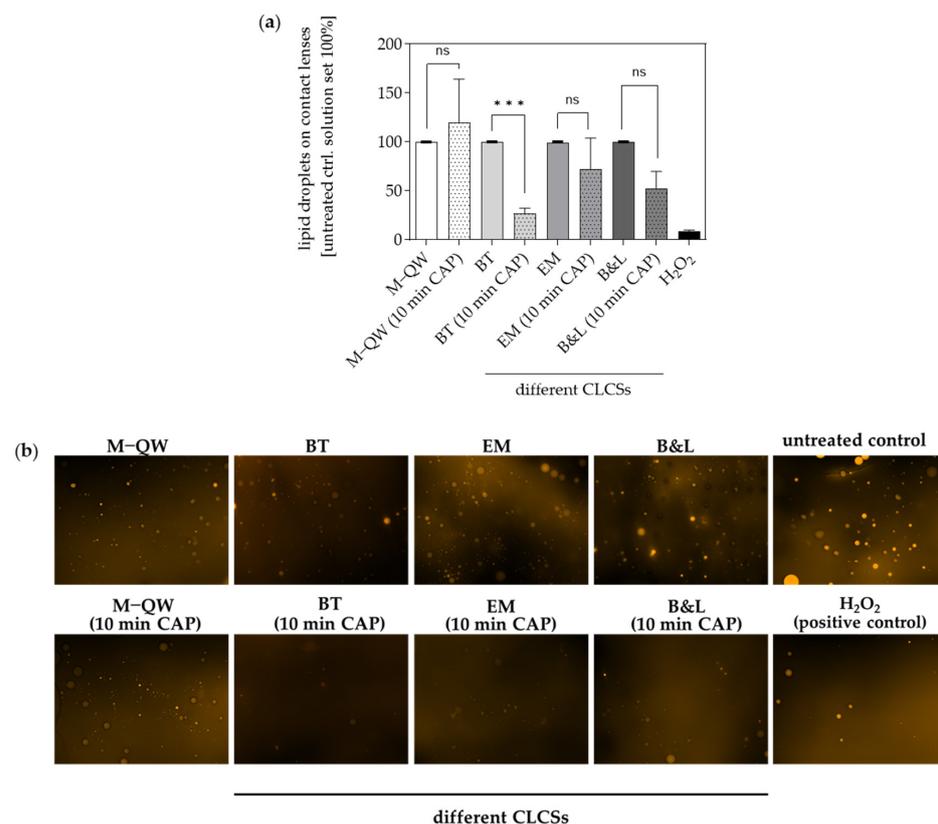


Figure 8. (a) Comparison of the cleaning effect of untreated M-QW, Bausch + Lomb’s Biotrue “All-in-One” solution (BT), MPG&E Handel und Service GmbH’s Eyemax24 solution (EM), and Bausch + Lomb’s Boston® One-Bottle Combination solution (B&L) with the respective CAP-treated solution on lipid-coated silicone hydrogel daily lenses. CAP treatment time of the different solutions was 10 min. H₂O₂, 3% was used as a positive control. Experiments were repeated $n = 4$ in duplicates and were summarized. Statistical analysis: Paired t test. *** $p < 0.001$, ns (not significant). (b) Exemplary representation of the cleaning effect after a 10 min CAP treatment and in the untreated state on lipid-coated silicone hydrogel daily lenses, observed using a fluorescence microscope at 5× magnification. Fluorescent dye used: Nile Red. Images were captured with an Axiocam MR in the wavelength range of 542–554 nm.

3.2.2. Effect of Plasma-Activated Solutions on the pH Value

To prevent irritation caused by contact lens solutions in lens wearers, the pH value of the cleaning solution plays an important role. It is similar to that of physiological tear fluid, which is 7.45 ± 0.16 . Several studies have reported changes in pH values following CAP treatment [44–46]; therefore, it was of interest whether the pH value after CAP treatment affects the CLCS (BT), DPBS, M-QW, and NaCl. CAP treatment of the four tested solutions had varying effects on their pH values. In the CLCS (BT) and DPBS, the pH remained relatively stable during treatment. The initial pH of untreated BT solution was slightly alkaline at 7.54 and decreased only slightly to 7.44 after 5 min of CAP treatment. In DPBS, the pH dropped by 0.28 units, from 7.18 to 6.9, shifting to a slightly acidic range. In contrast, stronger fluctuations were observed in M-QW and NaCl. The pH of M-QW decreased sharply from 6.26 to 2.95, and that of NaCl from 7.77 to 2.99 after 5 min of CAP treatment. Overall, all solutions showed a pH decrease following CAP exposure, which depended on the treatment dose. However, in CLCS (BT) and DPBS, buffering agents stabilize the pH, preventing strong acidification (Figure 9).

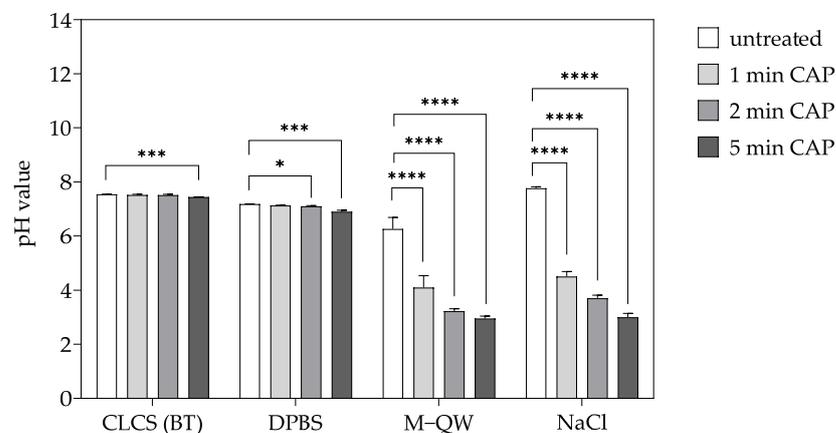


Figure 9. Effect of CAP treatment on the pH value of CLCS (Bausch + Lomb’s Biotrue “All-in-One” solution (BT)), phosphate-buffered saline (DPBS), Millipore water (M-QW), and physiological saline solution (NaCl). The CAP treatment times were 1 min, 2 min, and 5 min. Statistical significance refers to the respective untreated control. Statistical analysis: two-way ANOVA, Dunnett’s multiple comparisons test. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

4. Discussion

Contact lenses have greatly advanced vision correction, but complications such as corneal neovascularization, conjunctivitis, and keratitis remain risks [47,48]. Proper lens cleaning is crucial, yet studies have shown that the antimicrobial efficacy of many “All-in-One” CLCSs is often insufficient [49]. In recent years, disinfection using CAP has gained significant importance in the medical field [50]. The effects of CAP result from the synergistic interaction of various physical and chemical components, with RONS playing a key role. During CAP treatment, RONS can penetrate liquids, creating plasma-activated liquids (PALs) or plasma-activated solutions (PASs), whose chemical composition varies depending on the treated solution, the gas used, and the activation method. Previous studies have investigated how long these reactive species remain stable in different types of solutions. Most publications to date focus on plasma-activated water (PAW), though storage times and conditions vary greatly between studies [51–53]. In some cases, conclusions about the antibacterial efficacy of CAP-treated solutions are drawn based on the stability of the reactive species [54]. It is generally assumed that without an increased concentration of RONS, no antibacterial effect of the CAP-treated solution can be expected. This raised the question of whether CAP properties could also be applied to CLCSs to enhance their

antibacterial effectiveness. The Biotrue “All-in-One” solution (BT, Bausch + Lomb) was used as a representative CLCS. Since CLCSs typically use phosphate or borate buffers to maintain a physiological pH, this study also examined the effects of CAP treatment on RONS levels in phosphate-buffered saline (DPBS). Additionally, physiological saline (NaCl) and Millipore water (M-QW) were also tested. NaCl, commonly used for rinsing lenses, lacks antibacterial properties, so this study explored whether CAP could confer such effects.

In the short-term experiment, CAP treatment (1–5 min) caused an immediate dose-dependent increase in RONS (H_2O_2 , ROS, NO_2^- , NO_3^-) compared to untreated samples (Section 3.1.1). Long-term measurements (up to 4 weeks) showed that RONS levels generally remained elevated, especially in the buffered solutions (CLCSs and DPBS) (Section 3.1.2), indicating greater stability than in NaCl and M-QW. It is already known that RONS generally remain more stable in buffered solutions, as buffers prevent the strong acidification induced by CAP treatment and thus slow down pH-dependent degradation [55]. The stability of the pH value in CLCSs and DPBS was also confirmed in our study (Section 3.2.2). Next to the pH value, RONS stability in CAP-treated solutions also depends on storage time and temperature. While some report a rapid decline of H_2O_2 and NO_2^- within days [51], others observe stable levels over several weeks [52,53]. Temperature is a key factor in this—low temperatures (especially $-80\text{ }^\circ\text{C}$) help maintain RONS stability [54]. In this study, solutions were stored at room temperature in the dark, reflecting typical contact lens storage conditions. However, for future studies, it would be interesting to investigate how the stability of CLCSs after CAP treatment behaves at lower temperatures.

In addition, this study examined whether the CAP-induced increase in reactive species affected the antibacterial activity of the tested solutions against *E. coli*. *E. coli* was selected as a representative Gram-negative bacterium to evaluate the antibacterial efficacy of PASs, providing a clear baseline measure of antimicrobial activity against a common, well-characterized organism. Many studies in ophthalmology similarly employ this strain for their investigations [13,23,56], which facilitates comparison with existing literature and across experiments. Demonstrating a clear effect against *E. coli* establishes a proof of concept, but future work should include other clinically relevant organisms (such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*) to assess broad-spectrum applicability and organism-specific responses.

Testing three different CLCSs against *E. coli*, all showed some effect, but none achieved the 99.999% reduction needed for true disinfection. However, CAP treatment did not improve the antibacterial efficacy either, although enough RONS production and stability are present in CLCSs. Otherwise, CAP-treated M-QW and NaCl showed strong antibacterial effects against *E. coli*, achieving disinfection after just four hours with a 10-min CAP treatment. While CAP treatment can bolster antimicrobial efficacy in certain solutions, the benefit is not universal and can be mitigated by buffering and solution chemistry. Similar results were obtained by Chen et al. (2017) [57]. They observed that plasma-activated water and plasma-activated saline were much more effective than plasma-activated citrate in inactivating both *E. coli* and *S. aureus*. The plasma treatments acidified the water and saline, but the citrate buffer maintained its buffering capacity [57].

Longer exposure or extended CAP treatment further enhanced the antibacterial effect (Section 3.1.3). This results were supported by Tsoukou et al. (2021), showing that extending the contact time with the bacteria or increasing the CAP treatment duration enhanced the antibacterial effect of the CAP-activated solution [58]. According to Perinban et al. (2019), the antimicrobial properties of plasma-activated water result from the synergistic effects of the formation of RONS, its acidic pH, and high positive oxidation–reduction potential [59]. These factors can cause oxidative damage to the cell membrane, degradation of the cell

wall, cell shrinkage, leakage of cytoplasm, and DNA degradation, all of which contribute to its antibacterial activity [54]. However, this antimicrobial property of plasma-activated water does not apply to the CAP-activated CLCSs. The lack of interaction between an acidic pH and the increased concentration of RONS may explain why CAP treatment had no effect on the antibacterial activity of the CLCSs. Thus, an increase in RONS alone in CLCSs is not sufficient to enhance the antibacterial effect against *E. coli*.

In addition, contamination of contact lenses with bacteria, viruses, or fungi can be promoted by deposits on the lens surface, particularly lipid deposits [60]. These are mainly found on monthly and yearly lenses and can originate from external sources such as cosmetics or from endogenous lipids in the tear film [61]. This study tested the cleaning efficiency of different CAP-treated CLCSs on lipid-coated daily contact lenses compared to untreated samples (Section 3.2.1). All CAP-treated CLCSs showed improved lipid removal compared to their untreated counterparts, except for M-QW. The only difference between the buffered CLCSs and the unbuffered M-QW after CAP treatment was the pH, as already mentioned above. When the pH decreases, the concentration of positively charged hydrogen ions in the solution increases [62]. Since silicone hydrogel contact lenses are made of a negatively charged material [63], these hydrogen ions can bind to the lens surface, neutralizing its charge. This neutral state makes it easier for hydrophobic lipids, which do not dissolve well in polar solvents, to adhere to the lens surface. Consequently, the CAP-induced pH drop in M-QW leads to reduced cleaning efficiency against lipid-coated silicone hydrogel contact lenses. Unlike M-QW, the CLCSs contain various active ingredients that contribute to lipid removal. Since all solutions showed improved lipid-cleaning performance after CAP treatment, it can be assumed that CAP has a supportive effect on these cleaning components. In our experiments, only daily lenses were used, which are typically not cleaned but discarded after use. Therefore, it would be of great interest to investigate whether CAP treatment of the lens solutions could also enhance lipid cleaning in monthly or yearly lenses. Since CAP treatment of M-QW and NaCl shifts the pH into the acidic range, their use for contact lens cleaning is considered problematic. Some lenses are sensitive to pH changes and may alter their shape, leading to a reduction in diameter and posterior optical radius [64]. Moreover, acidic residues could enter the eye after cleaning, causing irritation. To prevent this, users could apply a neutralizing solution to the lens before wearing it, similar to the neutralization step used in effective peroxide cleaning systems, where catalase or sodium pyruvate solutions restore a neutral pH after H₂O₂ cleaning [31]. However, this would add an extra step and increase effort for lens wearers. While CAP-treated M-QW exhibited both increased RONS levels and a pH drop—resulting in antibacterial activity comparable to 3% H₂O₂—its acidic nature would make it unsuitable for direct ocular use due to the risk of irritation. Therefore, a combined approach may be promising: first using CAP-treated M-QW for bacterial removal, followed by CAP-treated CLCS to neutralize pH and enhance lipid cleaning. This two-step process could merge the advantages of both treatments, leading to more efficient lens cleaning and fewer contact lens-related complications.

5. Conclusions

In conclusion, CAP treatment demonstrated beneficial effects primarily in improving the cleaning efficiency of contact lenses, particularly in removing lipid deposits. This enhanced lipid removal likely reduces microbial adhesion and, consequently, the risk of infection-related contact lens complications such as infectious keratitis. However, CAP treatment did not significantly enhance the antibacterial activity of contact lens cleaning solutions against *E. coli*. This finding supports the idea that the antibacterial efficacy of CAP-

treated solutions depends not only on the increase in RONS but also on their interaction with a decrease in pH.

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Abbreviations

The following abbreviations are used in this manuscript:

CAP	Cold atmospheric plasma
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
RONS	Reactive oxygen and nitrogen species
PAS	Plasma-activated solution
PAL	Plasma-activated liquid
CLCS	Contact lens cleaning solution
M-QW	Milli-Q water
DPBS	Dulbecco's Phosphate Buffered Saline
<i>E. coli</i>	<i>Escherichia coli</i>
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
UVA	Ultraviolet A
UVB	Ultraviolet B
HSV	Herpes simplex virus
SMD	Surface-microdischarge
BT	Biotrue "All-in-One" solution
EM	Eyemax24 contact lens solution
B&L	Boston® One-Bottle Conditioning Solution
H ₂ O ₂	Hydrogen peroxide
NO ₂ [−]	Nitrite
NO ₃ [−]	Nitrate
CFU	Colony forming unit
DHR123	Dihydrorhodamine 123

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