

Electrochemically activated solutions: evidence for antimicrobial efficacy and applications in healthcare environments

R. M. S. Thorn · S. W. H. Lee · G. M. Robinson ·
J. Greenman · D. M. Reynolds

Received: 13 April 2011 / Accepted: 15 July 2011
© Springer-Verlag 2011

Abstract Due to the limitations associated with the use of existing biocidal agents, there is a need to explore new methods of disinfection to help maintain effective bioburden control, especially within the healthcare environment. The transformation of low mineral salt solutions into an activated metastable state, by electrochemical unipolar action, produces a solution containing a variety of oxidants, including hypochlorous acid, free chlorine and free radicals, known to possess antimicrobial properties. Electrochemically activated solutions (ECAS) have been shown to have broad-spectrum antimicrobial activity, and have the potential to be widely adopted within the healthcare environment due to low-cost raw material requirements and ease of production (either remotely or in situ). Numerous studies have found ECAS to be highly efficacious, as both a novel environmental decontaminant and a topical treatment agent (with low accompanying toxicity), but they are still not in widespread use, particularly within the healthcare environment. This review provides an overview of the scientific evidence for the mode of action, antimicrobial spectrum and potential healthcare-related applications of ECAS, providing an insight into these novel yet seldom utilised biocides.

Introduction

The use of biocides is an essential preventative control measure against the spread of nosocomial infections and multiple drug-resistant bacteria within hospital and other healthcare or

community settings. The general mechanism of action of biocides involves multiple target sites, making them highly efficacious as antimicrobials [1]. This reduces the risk of the development of resistance to these agents, compared to that associated with the use of antibiotics which usually only have a single target site [2]. Acquired resistance to antibiotics is of particular concern, as the number of antibiotic prescriptions is again increasing within the UK [3]. Frequent use of several existing biocides can cause respiratory or dermatological health problems in hospital workers [4–6], for example, following exposure to glutaraldehyde during the high-level disinfection of heat-sensitive equipment such as endoscopes [6]. Moreover, some have the potential to cause corrosion or damage to equipment [7]. Therefore, there is still the need to explore alternative biocides, particularly since there is evidence for resistance to existing biocidal agents [8, 9].

The use of electrolysis for disinfection has been employed for over 100 years [10], although it was not until the 1970s that the physicochemical properties of electrochemically activated solutions (ECAS) were extensively researched at the All-Russian Institute for Medical Engineering [11]. ECAS have since found numerous biocidal applications, for example, for potable water disinfection [12, 13] and within the food industry [14], and this is largely due to their high activity, use of cheap raw materials and ease of production. With the concern surrounding the emergence of antimicrobial resistance in the healthcare environment, the use of ECAS has been investigated for potential applications in clinical practice.

Generation of ECAS: the electrolytic cell and resultant stability

ECAS are produced via the electrolysis of a low mineral salt solution (the electrolyte) in an electrochemical cell

R. M. S. Thorn · S. W. H. Lee · G. M. Robinson · J. Greenman ·
D. M. Reynolds (✉)
Centre for Research in Biosciences, Department of Applied
Sciences, University of the West of England, Frenchay Campus,
Coldharbour Lane, Bristol BS16 1QY, UK
e-mail: Darren.Reynolds@uwe.ac.uk

(Fig. 1). When a direct current is applied (A), electrochemical processes at the material electrode surface transform the electrolyte (NaCl) into an activated ‘metastable’ state, exhibiting elevated chemical reactivity and resulting in the modification of molecular ionic structures [11]. Titanium (Ti) electrodes coated with porous layers of a metal oxide catalyst (e.g. RuO_2 , TiO_2 , SnO_2 , IrO_2) [15] are used due to improved characteristics of stability, selectivity, electrochemical reactivity, corrosion resistance and operating life of electrodes [16–18]. In the anodic chamber (Fig. 1), the continuously perfused salt (NaCl) solution reacts at the anode surface, producing mainly chlorine and oxygen, but also other reactive oxidants which are released into the bulk fluid. This is dependent on the redox reactions of strongly adsorbed electro-active water-derived intermediate molecular species [19–22], and a large scientific body of evidence now exists for these processes [15, 16, 23, 24]. This reaction is pH-dependent and (according to the Nernst equation) dictates which free form of chlorine is most prevalent within generated solution; Cl_2 , HClO or ClO [25, 26]. The exact physicochemical properties of the resulting anolyte (ECAS^a) is dependent on both the characteristics of the electrochemical cell and its operating parameters, although conditions conducive to a low pH (~2–3) and high oxidation-reduction potential (ORP) (above +800 mV) are usually sought. In the cathodic chamber (Fig. 1), hydrogen is generated, along with other reactive substances

(largely antioxidants), resulting in a decrease in the redox potential and an increased pH. Although these cathodic solutions (ECAS^c) have been used for the effective treatment of industrial effluents [15], they possess only limited antimicrobial activity [27, 28], and, hence, will not be the focus of this review.

The transformation of the electrolyte into a metastable state is not permanent. Upon the generation and recovery of ECAS^a, the chemical species present will shift spontaneously from this thermodynamically un-equilibrated condition to a stable non-active form, during what is known as the ‘period of relaxation’ [29]. The rate of relaxation, and, thus, the half-life of the active solution, is ECAS^a-specific [30]. However, the stability of ECAS^a can be improved by increasing the pH, since this shifts the chemical equilibrium towards non-volatile chlorine species; this has been shown experimentally [10, 31]. In contrast to the significant reductions in residual free chlorine, studies have shown that the pH, ORP, conductivity and chloride ion concentration levels are all relatively stable during short-term storage [31, 32], indicating that the oxidising potential of these solutions is largely retained.

Identification of the active antimicrobial agents within ECAS^a

ECAS^a characteristically have an ORP of +800 mV to +1,200 mV, creating an environment outside the working range of important microbial processes [33], including energy-generating mechanisms [34]. If immersed in these solutions, microorganisms will be exposed to powerful oxidants which will sequester electrons with high efficiency from microbial structural compounds, causing the rupturing of biochemical bonds and subsequent loss of function. Moreover, the high ORP environment is thought to create an unbalanced osmolarity between the ion concentrations in the solution and that within unicellular organisms, further damaging membrane structures [35]. This will cause increased membrane porosity, enabling oxidising moieties (present in excess in ECAS^a) to penetrate (via diffusion) into the cell cytoplasm, ultimately leading to the inactivation of intracellular protein, lipids and nucleic acid, rendering the cell non-functional.

It has been stated that ORP is more important than free chlorine content in terms of predicting the disinfectant potential of a given ECAS^a [36, 37], and this has been demonstrated experimentally by a number of researchers [38, 39]. The ORP of ECAS^a has been found to be inversely proportional to the pH [37], and that decreasing the pH increases the antimicrobial potential of ECAS^a, even if the residual chlorine levels are kept constant [40]. At low pH levels (~pH 2–5), HOCl will be the predominant

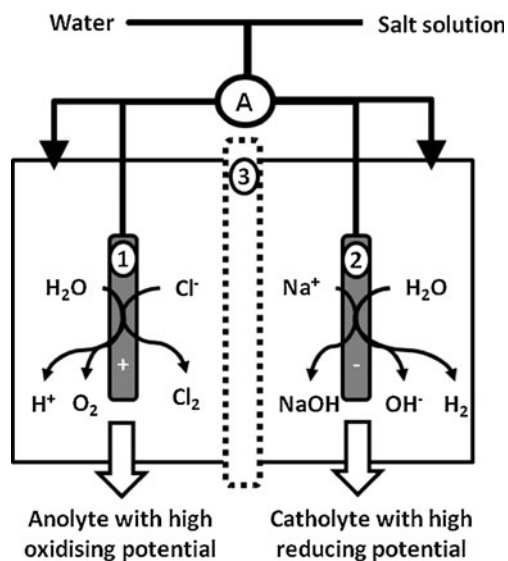


Fig. 1 Prototypical electrochemical cell used for generating electrochemically activated solutions (ECAS), comprising of two electrodes, an anode (1) and a cathode (2), separated by an ion-permeable exchange diaphragm (3). During operation, a salt solution is continuously perfused into both the anodic and cathodic chambers. The main general chemical reactions thought to occur at each electrode when a unipolar direct current is applied (amperage; A) are shown, with additional chemical transformations being dependent on the nature of the electrode material and specific electrolyte used

chlorine species present, leading many researchers to conclude that HOCl is the primary active agent present in acidic ECAS^a [10, 30, 40], being known to disrupt microbial structure [1] and the general cellular activity of proteins [2, 41, 42]. In addition, hydroxyl radicals (the strongest oxidising agent characterised) have also been detected within ECAS^a [15, 43–46], and it is likely that a combination of active moieties contribute to the antimicrobial efficacy of ECAS^a, creating an antimicrobial milieu that has been likened to that utilised by phagosomes to induce killing within phagocytic cells of the mammalian immune system [47].

The antimicrobial efficacy of ECAS^a is thought to be at least partially dependent on ‘non-specific’, short-lived, highly reactive oxidative moieties. These components will react with any organic compounds present within the environment, whether this is the desired target or not. In fact, the presence of organic loading has been shown to significantly reduce the antimicrobial potential of ECAS^a [48–50]. This is an important consideration in their application, since where a high organic load is likely, a high-strength solution (high ORP) or continual delivery will be required to maintain a high level of disinfection potential.

Efficacy of ECAS^a against specific microbial targets

The susceptibility assays used by different research groups to assess the antimicrobial efficacy of ECAS^a often vary, making direct comparisons problematic. However, if the quantitative studies within the literature are taken together, it is clear that ECAS^a is active against a broad spectrum of microorganisms (see Table 1), and these are described and discussed below.

Bacteria

Table 1 lists the aerobic, facultative and anaerobic bacterial species that have been shown to be susceptible to ECAS^a treatment during in vitro suspension tests. Extensive ECAS biocidal research has also been performed within Russia, Japan and China, although Table 1 only accounts for those studies published in English language journals. The kill rate (*k*) values for the various ECAS^a have been calculated using the viable count and time data points provided within each experimental study in order to account for the various experimental protocols (in particular, exposure time), since the kill rate is the key comparator for different biocidal experimental parameters [73]. However, within most studies, only a single contact and recovery time point was used. This is likely to account for the wide variation in kill rates observed, since, if only a single time point is taken

after a long incubation time, an apparently slower kill rate will be recorded, even if the majority of the killing occurred in the first few seconds of exposure. Very few studies have extensively characterised the antimicrobial kinetics of ECAS^a, and further research is required in this area. Nonetheless, the data is still representative of the spectrum of bactericidal activity of both acidic (pH 2–5) and neutral (pH 5–8) ECAS^a. It is clear that acidic ECAS^a has a broad spectrum of activity, including clinically relevant strains after only short exposure times (high kill rate), comparable to other regularly used disinfectants, including sodium hypochlorite, chlorhexidine gluconate, glutaraldehyde and benzalkonium chloride [74, 75]. The exact chemical composition of ECAS^a can vary, but one study comparing the antimicrobial activity of various commercial acidic ECAS^a solutions generated using either ‘pure’ (reverse-osmosed) or ‘local’ tap water showed no differences in activity [51]. More recently, there has been increased interest in pH-neutralised ECAS^a as an antimicrobial (e.g. Sterilox™ and Microcyn™), and although previous studies have shown antimicrobial efficacy to be a function of pH [30, 31, 40], these solutions have also shown broad-spectrum bactericidal activity [61, 62, 76] (Table 1). Neutralised ECAS^a are thought to benefit from increased biocompatibility and longer shelf life [76] and, hence, they may be more commercially valuable, having been proven to retain significant antimicrobial activity. However, few direct comparisons of acidic and pH-neutralised ECAS^a have been made (in particular, shelf life), precluding any meaningful conclusions, and further research is required to determine the effect of altering the pH alone on antimicrobial efficacy.

The high lipid content outer membrane and cell membrane bacterial structures are likely to be the primary ECAS^a target. ECAS^a are thought to sequester electrons from these structures, rendering them unstable, potentially allowing oxidants to penetrate into the cell cytoplasm, causing widespread oxidation and the inactivation of essential cellular processes [76]. Low pH could also sensitise the outer membrane of Gram-negative bacterial cells, enabling more efficient entry of hypochlorous acid [1]. It has been postulated that the high ORP of ECAS^a interferes with the cellular redox signalling pathways (e.g. glutathione disulphide–glutathione couple), causing cell permeabilisation, oxidative intra-cellular formation of disulphide bridges, consequent changes in protein structure and function, and, ultimately, cell lysis [39]. The effect of ECAS^a on bacterial cells has been directly observed using transmission electron microscopy [60, 66], atomic force microscopy [39, 77] and fluorescence microscopy [39], providing evidence of the direct effects on the bacterial cell envelope. Once within the bacterial cell, ECAS^a has been shown to cause the total destruction of chromosomal and

Table 1 Range of experimental kill rates determined for acidic (pH 2–5) and neutralised (pH 5–8) electrochemically activated solution anolyte (ECAS^a) against aerobic, facultative and anaerobic bacterial target species, bacterial spores, and eukaryotic cells, within in vitro suspension tests. Kill rates (*k*) are expressed as log₁₀ colony-forming

units (CFU) ml⁻¹ reduction per minute from the viable count and time data points provided within the literature, and, therefore, must be taken as the lowest estimates. Qualitative studies are reported where no quantitative data exist in the literature

Target organism	Experimental kill rates (<i>k</i>) of various ECAS ^a (log ₁₀ CFU ml ⁻¹ reduction per minute)	
	Acidic ECAS ^a	Neutralised ECAS ^a
Aerobic/facultative bacteria		
<i>Acinetobacter</i> spp.	+ [51]	10.0 [52]
<i>Actinobacillus actinomycetemcomitans</i>	+ [53]	+ [53]
<i>Aeromonas liquefaciens</i>	13.8 [54]	
<i>Alcaligenes faecalis</i>	13.6 [54]	
<i>Bacillus subtilis</i>	+ [10]	1.7 [55]
<i>Bacillus cereus</i>	2.3–5.9 [30, 54, 56]	
<i>Burkholderia cepacia</i>	34.5 [57]	
<i>Citrobacter freundii</i>	13.3 [54]	
<i>Campylobacter jejuni</i>	44.9 [58]	
<i>Escherichia coli</i>	1.4–37.4 [36, 38, 40, 54, 56, 57, 59, 60]	1.7–16.0 [48, 52, 59, 61]
<i>Enterobacter aerogenes</i>	16.0 [58]	10.0 [52]
<i>Enterococcus</i> spp.	14.5 [54]	3.5–15.4 [48, 52, 62]
VRE		3.5–10.0 [52, 62]
<i>Flavobacter</i> spp.	14.2 [54]	
<i>Haemophilus influenzae</i>		>10.0 [52]
<i>Helicobacter pylori</i>	+ [63]	3.50 [62]
<i>Lactobacillus</i> spp.		4.4–5.0 [55]
<i>Legionella pneumophila</i>		8.0 [64]
<i>Listeria monocytogenes</i>	1.3–16.3 [36, 40, 56, 65]	
<i>Klebsiella</i> spp.		10.0 [52]
<i>Micrococcus luteus</i>		10.0 [52]
<i>Mycobacterium</i> spp.	+ [66, 67]	3.5–5.1 [57, 63]
<i>Proteus</i> spp.	14.0 [54]	10.0 [52]
<i>Pseudomonas aeruginosa</i>	14.1–37.4 [54, 57, 68]	8.0–16.0 [48, 52, 64]
<i>Salmonella</i> spp.	6.1–8.0 [59, 69]	5.2–16.0 [59, 61, 65]
<i>Serratia marcescens</i>	37.4 [57]	10.0 [52]
<i>Staphylococcus</i> spp.	3.7–37.4 [54, 57, 59, 60, 69]	3.9–16.0 [55, 59, 61, 64, 69]
MRSA	28.8–37.4 [57, 68]	13.4 [48]
MRSE		3.2 [55]
<i>Streptococcus</i> spp.	+ [51, 53]	3.8–5.0 [55]
<i>Xanthomonas maltophilia</i>	+ [51]	
Anaerobic bacteria		
<i>Actinomyces</i> spp.	+ [53]	2.9 [55]
<i>Bifidobacterium bifidum</i>		5.0 [55]
<i>Bacteroides fragilis</i>		10.0 [52]
<i>Eubacterium lentum</i>		3.0 [55]
<i>Fusobacterium nucleatum</i>	+ [53]	2.9 [55]
<i>Peptococcus niger</i>		4.2 [55]
<i>Peptostreptococcus anaerobius</i>		4.1 [55]
<i>Prevotella melaninogenica</i>	+ [53]	5.8 [55]
<i>Porphyromonas</i> spp.	+ [53]	3.5 [55]
<i>Prevotella loeschii</i>	+ [53]	5.5 [55]
<i>Propionibacterium acnes</i>		4.6 [55]
<i>Veillonella parvula</i>		4.7 [55]

Table 1 (continued)

Target organism	Experimental kill rates (<i>k</i>) of various ECAS ^a (log ₁₀ CFUml ⁻¹ reduction per minute)	
Bacterial spores		
<i>Bacillus anthracis</i>		0.2 [70]
<i>Bacillus atrophaeus</i>	3.7 [68]	0.4–2.0 [52, 61]
<i>Bacillus cereus</i>	1.32–6.98 [54, 56]	
<i>Bacillus subtilis</i>	0.9 [66]	1.0–15.0 [48, 71]
<i>Clostridium difficile</i>	16.3 [68]	2.0 [62]
<i>Clostridium perfringens</i>		0.04 [72]
<i>Streptomyces</i> spp.	+ [28]	+ [28]
Eukaryotes		
<i>Aspergillus</i> spp.	1.48 [46]	5.25 [46]
<i>Candida</i> spp.	3.5 [62]	3.5–16.0 [48, 61, 62, 64]
<i>Cryptosporidium parvum</i> oocysts		* [72]
Various environmental fungi	+ [70]	

VRE: vancomycin-resistant *Enterococcus*; MRSA: methicillin-resistant *Staphylococcus aureus*; MRSE: methicillin-resistant *Staphylococcus epidermidis*

+Qualitative study only

*1.3 log reduction of oocyst infectivity in 1 h

plasmid DNA, RNA and proteins when analysed using both sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) [60, 78, 79] and a restriction fragment length polymorphism pattern (RFLP) assay [66]. However, it is likely that cell death/lysis results from the early events involved with cell membrane disruption and consequent potassium leakage from the cell.

Bacterial spores

Bacterial spores are innately more resistant to antimicrobial treatment due to various physiological factors [80, 81]. ECAS^a (in common with all known biocides) have shown reduced efficacy against spores compared to vegetative cells, although it is evident from the literature that significant sporicidal activity has still been proven in vitro for both acidic and neutralised ECAS^a (see Table 1). One study using acidic ECAS^a in conjunction with a kinetic assay showed spores of *Bacillus atrophaeus* to be significantly more resistant than *Clostridium difficile*, although both test strains were reduced to minimum detection levels within 90 s of exposure [68]. pH-neutralised ECAS^a have been found to have greater sporicidal activity than some existing biocides, e.g. glutaraldehyde [51], 70% ethanol or 70% isopropanol [61]. Moreover, a pH-neutralised ECAS^a was found to have a significant sporicidal activity against the potential bio-terrorism agent *Bacillus anthracis*, equivalent to that of 5% calcium hypochlorite advocated by the U.S. military for the decontamination of spores on skin or surfaces [82]. The authors concluded that, due to the toxic and corrosive nature that existing agents posed to human

health, pH-neutralised ECAS^a may offer a real practical alternative.

One study using *Bacillus subtilis* knock-out mutant spores showed that ECAS^a was not targeting DNA or germination as its primary mechanism of action, as evidenced by the observation of germination-specific events even in killed spores [71]. It was postulated that ECAS^a oxidatively modifies the inner membrane, targeting proteins and unsaturated fatty acids, and that, since this membrane structure will eventually become the outgrowing spores' cell membrane, this ultimately renders the spore non-functional [71].

Biofilms

Microorganisms are now known to form resistant biofilm structures [83, 84], which are thought to have evolved as a tenacious survival strategy [85]. Although these structural communities are undoubtedly ubiquitous in nature, few experimental studies have been performed to specifically investigate the sensitivity of these 'antimicrobial-tolerant' communities to ECAS^a. The effective removal of mature *Pseudomonas aeruginosa* biofilms from the surface of glass and stainless steel after treatment with either acidic or neutralised ECAS^a has been shown in vitro by light and electron microscopy [86]. In addition, removal of the extracellular matrix of both *Escherichia coli* and sulphate-reducing bacterial biofilms has been observed using atomic force microscopy in vitro, after treatment with acidic ECAS^a [77], indicating its possible application as an antibiofouling agent. *Listeria monocytogenes* biofilms, formed on the surface of stainless steel coupons, were also shown to be sensitive to a

neutralised ECAS^a, which elicited a 9 log₁₀ reduction after 5 min of treatment [86]. Numerous other studies have looked at the inactivation of surface-associated bacterial cells subsequent to ECAS^a treatment, and have shown significant activity against *Staphylococcus aureus* (including methicillin-resistant *S. aureus* [MRSA]), *Enterococcus faecalis*, *E. coli*, *L. monocytogenes*, *Acinetobacter baumannii*, *Helicobacter pylori* and *Mycobacterium* spp. [7, 27, 63, 69, 87]. Biofilms are of particular concern in the oral cavity, as these polymicrobial communities can contribute to periodontal disease states and ECAS^a have been shown to be effective at removing necrotic dentine and pulp tissue, as well as microorganisms from tooth surfaces [88], which would otherwise likely lead to biofilm development associated with oral diseases.

The antimicrobial activity of ECAS^a is dependent on highly reactive non-specific oxidants (as previously described), and these active moieties are almost certainly competitively quenched by the high levels of organic load present within a biofilm structure (particularly the extracellular polymeric matrix). Therefore, a sufficient concentration and exposure time would be required to reach cells deeper within the biofilm architecture. In fact, one author postulated that hydroxyl radicals present in ECAS^a may cause the collapse of the highly structured hydrated biofilm matrix by removing hydrogen ions (through oxidation), exposing deeper biofilm cells to antimicrobial agents [89]. Collectively, the literature supports the potential use of ECAS^a against biofilms structures, but further research is required in this area to elucidate the kinetics and characterise appropriate treatment regimens.

Eukaryotes

ECAS^a is a broad-spectrum, non-selective biocide, hence, it has been shown to effectively inactivate certain pathogenic eukaryotic species (see Table 1) and is thought to damage cellular functional structures [46]. Of particular note is its efficacy against *Cryptosporidium parvum*, a waterborne pathogen that has previously been shown to be resistant to standard chlorine treatment [90]. pH-neutralised ECAS^a showed significant activity against *C. parvum* oocysts in contrast to little or no activity using a free chlorine solution [72]. Although few eukaryotic pathogens have been tested for their sensitivity to ECAS^a (see Table 1), it is evident from a study using environmental fungal species that it has significant broad-spectrum antifungal potential [70]. The sensitivity of eukaryotic cells to ECAS^a raises concerns regarding mammalian toxicity, which is considered later.

Microbial toxins

The ability of ECAS to inactivate pre-formed bacterial toxins has been investigated using staphylococcal

enterotoxin-A (SEA) [91]. This toxin is classically heat-stable and resistant to treatment with strong acid and alkali; nonetheless, significant inactivation was recorded when ECAS^a was present in excess [91]. In-depth analysis found that the immunoreactive site of SEA was denatured (even in the presence of organic loading) and that extensive peptide fragmentation occurred with accompanying loss of amino acid content [91]. The ability of ECAS^a to inactivate fungal toxins has been investigated using the aflatoxin of *Aspergillus parasiticus* and a significant reduction in the mutagenic potential of this aflatoxin was measured using a conventional Ames test [44]. The mode of action was postulated to be mediated by free-radical reactions, since the presence of radical scavengers (mannitol and thiourea) significantly reduced the efficacy of ECAS^a in destroying aflatoxin [44]. These isolated reports of the ability of ECAS^a to inactivate microbial toxins indicate its efficacy not only at killing whole microorganisms, but also deactivating or degrading their virulence factors.

Viruses

Chemical disinfection is seen as a valuable tool in limiting the environmental spread of infectious virions. Numerous studies have demonstrated the virucidal activity of ECAS^a against a broad range of targets [48, 51, 61, 92–97], comparable to that of other biocidal agents [92]. Most methodologies expose virus particles in suspension to ECAS^a in the presence/absence of organic loading, whereby ECAS^a reduces the number of viable virus particles as measured by cytopathic effects of the target virions in subsequently infected cell lines [48, 51]. An immunoassay has been used to assess ECAS^a-treated hepatitis B virus (HBV) surface antigen (in the absence of an appropriate whole-cell bioassay) and a significant concentration-dependent reduction in the measured antigenicity was observed [93]. The authors postulated that this was indicative of a reduction in the infectivity of human HBV [93] and this is supported by the finding that ECAS^a reduced the infectivity of a hepatitis B surrogate, duck hepatitis B virus, indicating the efficacy of ECAS^a against hepadnaviruses [92]. Similarly, the ECAS^a treatment of the norovirus surrogate bacteriophage MS2 was shown to significantly reduce infectivity, although significantly longer exposure times were required for surface-associated virions, presumably due to reduced accessibility of the active moieties [94]. It was, therefore, suggested that carrier/surface tests are more appropriate when testing the virucidal activity of environmental biocides. Fogged ECAS^a has been found to significantly reduce the surface levels of both human norovirus and surrogate viruses, as detected by reverse transcriptase polymerase chain reaction [94], and both acidic and neutralised ECAS^a have shown

significant activity against human immunodeficiency virus (HIV), even when infectious particles are pre-adsorbed onto an inanimate surface [61, 95]. Since viruses do not have cell walls, it has been postulated that the mode of action is likely to be the inactivation of surface protein, destruction of the viral envelope, inactivation of viral enzymes or the destruction of viral nucleic acid [92, 93], collectively eradicating their potential infectivity. In support of this theory, at least some ECAS^a components have been shown to penetrate the viral envelope [93].

Potential toxicity

The goal of disinfection is to reduce potentially pathogenic microbial populations to safe levels. In the clinical environment where contact with humans is either likely (e.g. cleaning products) or inevitable (e.g. topical treatments), agents must not be hazardous or toxic to living tissue, according to their particular application and in-use concentrations. A large scientific body of evidence now exists indicating the safety and non-toxicity of ECAS^a [11]. A single-dose and 28-day repeated dose oral toxicity study of ECAS^a in rats found no evidence of adverse effects [98], and mice given free access to ECAS^a as drinking water for 8 weeks showed no toxic side effects [99]. Moreover, no toxicity has been observed using in-use concentrations during acute oral toxicity tests (LD₅₀) upon application to mucous membranes, in accumulation irritation tests or in sensitisation tests, indicating its biocompatibility [52, 92, 93, 100–102]. In fact, the observed biocompatibility of ECAS^a has often been determined at relatively high exposure levels, in comparison with the anticipated low levels that would be used in the real clinical situation [103]. The incubation of ECAS^a with human cell lines *in vitro* has shown more mixed results, where some studies have shown no effect [102, 104], while others have shown significant cytotoxicity [105–107], although usually to a lesser degree than other commonly used biocides [104–106]. However, *in vitro* cytotoxicity is not always indicative of toxicity when used *in vivo*, as has been observed previously [105]. *In vitro* mutagenicity studies have failed to find any evidence of ECAS^a induced genotoxicity, using either the Ames test [102] or the genotoxicity micronucleus test [52], indicating its safe usage. Moreover, a recent wide-ranging toxicity study on a neutralised ECAS^a found that it did not degrade nucleic acids or induce oxidative damage in dermal fibroblasts *in vitro* [47]. This study led the authors to conclude that ECAS^a did not target cell nuclei, produced only limited damage to cell membranes and did not induce DNA oxidation or accelerated ageing [47]. It is also worth noting that ECAS^a presents no environmental hazard, since it slowly reverts to salt water during the period of chemical

relaxation, and is effectively inactivated by organic matter when present in trace amounts.

Potential corrosiveness of ECAS

The potential for biocides to cause material corrosion must be investigated before being widely used to disinfect inanimate surfaces. ECAS^a have highly oxidative properties, hence, this is of particular concern if ECAS^a are to be used as broad-spectrum multipurpose disinfectants. Few scientific studies have been performed to specifically investigate this, although one study has shown that low-level metal corrosion (stainless steel) and synthetic resin degradation occurred during a 36-day incubation with various acidic ECAS^a (replenished daily) [51]. This was described as “surface corrosion undetectable to the naked eye” in comparison to the strong corrosion exhibited by a 0.1% sodium hypochlorite solution also tested as a comparison over the same 36-day exposure time. It was concluded that these experimental results, coupled with their observations of the use of ECAS^a within a clinical setting for >3 years (with no observed corrosion problems), demonstrated a low risk of ECAS^a-mediated corrosion. A more recent study has shown that acidic ECAS^a had no adverse effect on stainless steel surfaces (after 8 days of contact), but significant corrosion was seen for carbon steel and, to a lesser extent, on copper and aluminium surfaces [108], likely to be due to the known susceptibility of these materials to oxidising agents (particularly chloride ions). Interestingly, this study showed how corrosion could be limited by using neutralised ECAS^a [108], highlighting the importance of testing the corrosive nature of specific ECAS^a within the real-world situation where they are to be applied.

Antimicrobial applications in healthcare: evidence of efficacy

Although initially used in an empirical manner, a large scientific body of evidence now exists from investigating the comparable merits of using ECAS^a against the ‘best available treatment’ within various medical disciplines:

(i) Treatment and prevention of wound infection

The use of targeted antibiotic therapy is essential in wound care for the treatment of known wound infections (e.g. *S. aureus*), but, due to the rise of antimicrobial resistance, the general use of broad-spectrum antibiotics is being restricted. Therefore, although prophylactic antibiotics are still used in surgery, broad-spectrum biocides are finding increased usage in antiseptic scrubs,

as wound irrigants, as well as for incorporation into wound-dressing products. Acidic ECAS^a used twice-daily to wash infectious defects or ulcers (15 case study participants) was shown to reduce bacterial infections and aid debridement, often where traditional treatment was found to be ineffective [109]. In another case study-based trial, seven patients with peritonitis or intraperitoneal abscesses underwent twice-daily ECAS^a lavage procedures, and were found to revert to a microbial-negative state within 3–7 days [33]. ECAS^a treatment significantly improved the survival rates within a rodent in vivo burn wound model infected with *P. aeruginosa*, along with a reduction in serum endotoxin levels [110]. Moreover, acidic ECAS^a have been found to promote re-epithelisation (in an in vivo burn wound model), increasing the proliferation of lymphocytes and macrophages associated with dense collagen deposition [111]. The clinical evidence for the use of ECAS^a is largely based on small-scale case studies, but it has shown promise in reducing bacterial infections in burn wounds [112], for the treatment of refractory chronic ulcers [109], as well as synergistic necrotising infections [113], and a neutralised ECAS is now commercially available specifically for the treatment of wounds (Dermacyn, Oculus Innovative Sciences, Petaluma, CA, USA [114]). Neutral ECAS^a have been shown to significantly increase healing rates and reduce pain levels in recalcitrant venous leg ulcers [115, 116], improve healing outcomes in diabetic foot ulcers [47, 117, 118], shown potential applications in advanced wound care in combination with negative pressure therapy [119] and have been shown to be more effective than povidone–iodine in treating diabetic foot ulcers [120]. Moreover, a recent randomised controlled trial using a daily instillation of pH-neutralised ECAS^a within wound dressings for the management of wide postsurgical lesions of the diabetic foot found it to significantly improve healing rates, while significantly reducing the bacterial load (compared to the control treatment, povidone–iodine) with no reported adverse effects [121].

ECAS^a is thought to help promote healing by reducing the bacterial load, enhancing local blood supply, accelerating neovascularisation, reducing inflammation and producing an environment hostile to opportunistic pathogens [117]. In addition, it is also thought to reduce odour levels by reacting with putrefying necrotic tissue [47]. ECAS^a have been trialled as a preventative therapeutic solution for postoperative infection [122] and reductions in the rates of infection (including those attributable to MRSA) have been observed [123]. ECAS^a have also shown potential for use in disinfecting the ocular surface [105] and the treatment of inflammatory acne lesions [124], providing

further evidence of their anti-inflammatory activity. Collectively, ECAS^a have shown promise in providing effective infection control with minimal damage to the regenerating host tissue. However, due to the predominately small-scale case study-based nature of the trials conducted, the evidence must be viewed with caution, and large-scale trials will be required before wide-spread usage is likely to be accepted.

(ii) Treatment and prevention of periodontal disease

The dental community has long sought adequate antimicrobial products to try to control proliferation of the indigenous oral microflora, particularly during dental surgery when the barrier functions of the host are often compromised. Early studies have shown that ECAS^a is capable of removing the smear layer from root canals in vivo [88] and was as effective as chlorhexidine in inhibiting plaque formation in human subjects [125]. This confirmed that the in vitro activity of ECAS^a against oral microorganisms (see Table 1) was also observed in vivo, whereby acidic, neutralised and low available chlorine concentration ECAS^a have all been shown to be active against cariogenic bacteria [53]. A tooth irrigant should not only possess antimicrobial activity, but also provide mechanical flushing action and dissolve remnants of organic tissue, ideally without damaging surrounding healthy tissue. One pilot study using extracted teeth showed that the combined application of ECAS^a and ECAS^c could be used as an effective root canal cleaning solution, comparable to sodium hypochlorite, as visualised by ESEM [126]. However, the antimicrobial efficacy of ECAS^a compared to the ‘best available treatment’ has been questioned by some authors, who have shown it to have only limited activity compared to other in-use treatments, e.g. EDTA or NaOCl [27, 127, 128]. The discrepancies in the literature are likely due to the method of delivery, and this has been suggested to be the critical treatment factor [128, 129].

(iii) Medical device disinfection

One of the earliest clinical applications of ECAS^a was for disinfecting medical equipment, and there are many studies showing its efficacy at disinfecting endoscopy equipment [48, 62, 63, 102], including bronchoscopes [7] and haemodialysis equipment [51]. However, due to the low levels of corrosion associated with ECAS^a use (see the section titled [Potential Corrosiveness of ECAS](#)), one UK endoscope manufacturer has stated that its warranty is void if ECAS^a is used to disinfect them [130]. Interestingly, one study showed clinical bacterial isolates to be more resistant than ‘laboratory’ strains to ECAS^a treatment [48], highlighting the need to include targets relevant to the in-use application of this technology during

research and development. One concern in dental environments is the microbial contamination of dental unit water lines, which, if inadequately disinfected, may harbour polymicrobial biofilms containing potentially pathogenic organisms. Since ECAS^a have been shown to be effective at removing biofilms, it is perhaps not surprising that they have proven to be useful in reducing the bacterial load of these medical devices [89, 131]. The fast-acting nature of these disinfectants reduces required contact and exposure times, potentially enabling high-throughput disinfection of medicinal equipment, often an important factor for repeat-use medical apparatus.

(iv) Environmental decontamination

Potentially pathogenic microorganisms can persist within the healthcare environment not only via direct transmission from patient to patient, but also through survival on the diverse array of inanimate surfaces present. Although viruses may only persist for short periods, bacteria can survive for months using the low-level nutrient sources available [132] or can revert to a dormant state (e.g. spores) until they are exposed to conditions conducive to growth. The potential use of ECAS^a to disinfect inanimate surfaces has been shown experimentally [69], and fogged ECAS^a has shown activity against MRSA, *Acinetobacter baumannii* and norovirus [94, 133]. This could have relevant applications in decontaminating large spaces (e.g. hospital wards), and targeted use of fogged/aerosolised ECAS^a may help control healthcare-associated infection outbreaks. A neutralised ECAS^a has been shown to be effective at reducing bacterial levels in industrial cooling towers in accordance with the UK Health and Safety Commission (HSC) Approved Code of Practice and Guidance (ACOP) [134]. Since several hospital outbreaks of *Legionella pneumophila* are thought to have originated from contaminated cooling towers [135], this demonstrates the wide range of applications where ECAS^a may help to control the microbial bioburden within global healthcare environments. Interestingly, ECAS^a have also been investigated for their application in hand washing, but, although showing significant reductions in bacterial numbers compared to washing in water, have shown only limited activity when compared to existing agents [136–139].

Discussion

The introduction of the Biocidal Product Directive 98/8/EC, and subsequent ongoing 10-year review of all existing and

emerging biocidal agents, has significantly reduced the number of biocide products available on the European market (as well as limiting the introduction of new or novel agents), largely due to the prohibitive costs involved with gaining approval [140]. Therefore, it is imperative that medical, government, industrial and academic institutions collaborate in order to help develop or validate the use of novel biocidal products in maintaining effective bioburden control, especially within the healthcare environment. The advantages and disadvantages of ECAS^a as applied to its potential usage within a healthcare setting are listed in Table 2. Although there is an initial expenditure on the electrolytic cell, once installed, the production of active solutions is cheap due to the relative abundance of raw materials (H₂O and NaCl). Due to on-site generation and low operator skill requirements, high ECAS^a production rates can be achieved, and this negates the need for the transport or storage of biocidal chemicals. The broad-spectrum antimicrobial activity of ECAS^a enables high-level disinfection as defined by the Centers for Disease Control and Prevention (CDC) [141], and their favourable biocompatibility means that ECAS^a are ideally suited as both an environmental decontaminant and in the control or treatment of skin surface or mucous membrane infections. ECAS^a do have their limitations. In general, they cannot be stored for long periods and the potency of ECAS^a will be dependent on the efficiency of the generator cell. In addition, acidic ECAS solutions can cause low levels of corrosion to some materials [108], and its antimicrobial activity quickly diminishes on contact with organic substrates [49]. Therefore, it is important that, for every new application, the actual ECAS^a disinfection or treatment regimen is appropriately designed and supported by a scientific body of evidence to validate its usage. For example, for effective disinfection in the presence of high

Table 2 General advantages and disadvantages of ECAS^a as applied to its potential usage within a healthcare setting

Advantages	Disadvantages
<ul style="list-style-type: none"> • Broad-spectrum antimicrobial activity • Rapid disinfection time 	<ul style="list-style-type: none"> • Initial expenditure on generator
<ul style="list-style-type: none"> • Inexpensive • Easily accessible raw materials • On-site or in-situ generation • Requires little operator skill • Limited toxicity • Environmentally compatible • Evidence of being anti-inflammatory 	<ul style="list-style-type: none"> • Generator servicing and maintenance • Limited shelf life • Inactivated by organic loading • Acidic ECAS^a can be corrosive

organic loads, repeated or continual delivery of ECAS may be required. However, characteristics undesirable for one application may be advantageous in another, and the organic quenching of ECAS^a activity is likely to underpin its low toxicity, thereby, promoting its usage as a skin and mucous membrane antiseptic.

The effective use of disinfectants within the healthcare environment almost certainly provides widespread protection to both healthcare practitioners and patients against possible contamination with potentially pathogenic organisms. Moreover, with the concern over antibiotic-resistant nosocomial infections, new or novel broad-spectrum antimicrobial treatments are in high demand. ECAS have been studied for many years and have been found to be highly efficacious biocidal agents, with increasing reports of their effectiveness in real-world applications; however, they are still not in widespread use, particularly within the healthcare environment. The paucity of wide-ranging clinical trials is likely to be a contributing factor, but recent guidelines do recognise the potential of ECAS^a for disinfection and sterilisation in healthcare facilities [141]. Further application-focussed research and development is required if ECAS are to replace established methods of disinfection and antiseptics, and find common usage within healthcare environments.

Acknowledgements The authors would like to thank Dann Turner for his critical assessment of the manuscript.

References

- Denyer SP (1995) Mechanisms of action of antibacterial biocides. *Int Biodeterior Biodegrad* 36(3–4):227–245
- McDonnell G, Russell AD (1999) Antiseptics and disinfectants: activity, action, and resistance. *Clin Microbiol Rev* 12(1):147–179
- Health Protection Agency (HPA) (2008) Antimicrobial resistance and prescribing in England, Wales and Northern Ireland. HPA, London
- Di Stefano F, Siriruttanapruk S, McCoach J, Burge PS (1999) Glutaraldehyde: an occupational hazard in the hospital setting. *Allergy* 54:1105–1109
- Sobaszek A, Hache JC, Frimat P, Akakpo V, Victoire G, Furon D (1999) Working conditions and health effects of ethylene oxide exposure at hospital sterilization sites. *J Occup Environ Med* 41(6):492–499
- Rideout K, Teschke K, Dimich-Ward H, Kennedy SM (2005) Considering risks to healthcare workers from glutaraldehyde alternatives in high-level disinfection. *J Hosp Infect* 59(1):4–11
- Middleton AM, Chadwick MV, Sanderson JL, Gaya H (2000) Comparison of a solution of super-oxidized water (Sterilox) with glutaraldehyde for the disinfection of bronchoscopes, contaminated. *J Hosp Infect* 45(4):278–282
- Fraiese AP (2002) Biocide abuse and antimicrobial resistance—a cause for concern? *J Antimicrob Chemother* 49(1):11–12
- Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) (2009) Assessment of the antibiotic resistance effects of biocides. SCENIHR, European Commission
- Nakagawara S, Goto T, Nara M, Ozawa Y, Hotta K, Arata Y (1998) Spectroscopic characterization and the pH dependence of bactericidal activity of the aqueous chlorine solution. *Anal Sci* 14:691–698
- Prilutsky VI, Bakhir VM (1997) Electrochemically actuating water: anomalous characteristics, mechanism of biological action. VNIIMT, Moscow
- Kraft A (2008) Electrochemical water disinfection: a short review. *Platinum Metals Rev* 52(3):177–185
- Barratt LP, Lloyd BJ, Graham JD (1990) Comparative evaluation of two novel disinfection methods for small-community water treatment in developing countries. *Aqua* 39(6):396–404
- Huang Y-R, Hung Y-C, Hsu S-Y, Huang Y-W, Hwang D-F (2008) Application of electrolyzed water in the food industry. *Food Control* 19(4):329–345
- Cai Z (2005) Characterisation of electrochemically activated solutions for use in environmental remediation. Ph.D. Thesis. University of the West of England, Bristol
- Trasatti S (1987) Progress in the understanding of the mechanism of chlorine evolution at oxide electrodes. *Electrochim Acta* 32(3):369–382
- Trasatti S (2000) Electrocatalysis: understanding the success of DSA[®]. *Electrochim Acta* 45(15–16):2377–2385
- Evdokimov S (2000) Mechanism of chlorine evolution-ionization on dimensionally stable anodes. *Russ J Electrochem* 36(3):227–230
- Burke LD, O'Neill JF (1979) Some aspects of the chlorine evolution reaction at ruthenium dioxide anodes. *J Electroanal Chem* 101(3):341–349
- Erenburg R, Krishtalik L, Rogozhina N (1984) pH effect on chlorine reaction kinetics on ruthenium titanium oxide anode. *Elektrokhimiya* 20:1183–1190
- Boggio R, Carugati A, Lodi G, Trasatti S (1985) Mechanistic study of Cl₂ evolution at Ti-supported Co₃O₄ anodes. *J App Electrochem* 15(3):335–349
- Trasatti S (1991) Physical electrochemistry of ceramic oxides. *Electrochim Acta* 36(2):225–241
- Devilliers D, Mahe E (2007) Modified titanium electrodes. In: Nunez M (ed) *New trends in electrochemistry research*. Nova Science Publishers, Inc., New York, pp 1–60
- Tomcsányi L, De Battisti A, Hirschberg G, Varga K, Liszi J (1999) The study of the electrooxidation of chloride at RuO₂/TiO₂ electrode using CV and radiotracer techniques and evaluating by electrochemical kinetic simulation methods. *Electrochim Acta* 44(14):2463–2472
- Stoner GE, Cahen GL Jr, Sachyani M, Gileadi E (1982) The mechanism of low frequency a.c. electrochemical disinfection. *Bioelectrochem Bioenerget* 9(3):229–243
- McPherson LL (1993) Understanding ORP's role in the disinfection process. *Water Eng Manage* 140(11):29–31
- Gulabivala K, Stock CJR, Lewsey JD, Ghori S, Ng Y-L, Spratt DA (2004) Effectiveness of electrochemically activated water as an irrigant in an infected tooth model. *Int Endod J* 37(9):624–631
- Hotta K, Kawaguchi K, Saitoh F, Saito N, Suzuki K, Ochi K, Nakayama T (1994) Antimicrobial activity of electrolyzed NaCl solutions: effect on the growth of *Streptomyces* spp. *Antinomycetologica* 8(2):51–56
- Petrushanko IY, Lobyshev VI (2001) Nonequilibrium state of electrochemically activated water and its biological activity. *Biofizika* 46(3):389–401
- Len SV, Hung YC, Erickson M, Kim C (2000) Ultraviolet spectrophotometric characterization and bactericidal properties of electrolyzed oxidizing water as influenced by amperage and pH. *J Food Prot* 63:1534–1537
- Len SV, Hung YC, Chung D, Anderson JL, Erickson MC, Morita K (2002) Effects of storage conditions and pH on chlorine loss in

- electrolyzed oxidizing (EO) water. *J Agric Food Chem* 50 (1):209–212
32. Hsu S-Y, Kao H-Y (2004) Effects of storage conditions on chemical and physical properties of electrolyzed oxidizing water. *J Food Eng* 65(3):465–471
 33. Inoue Y, Endo S, Kondo K, Ito H, Omori H, Saito K (1997) Trial of electrolyzed strong acid aqueous solution lavage in the treatment of peritonitis and intraperitoneal abscess. *Artif Organs* 21(1):28–31
 34. Kumon K (1997) What is functional water? *Artif Organs* 21 (1):2–4
 35. Chittoria RK, Yootla M, Sampatrao LM, Raman SV (2007) The role of super oxidized solution in the management of diabetic foot ulcer: our experience. *Nepal Med Coll J* 9:125–128
 36. Venkitanarayanan KS, Ezeike GO, Hung Y-C, Doyle MP (1999) Efficacy of electrolyzed oxidizing water for inactivating *Escherichia coli* O157:H7, *Salmonella enteritidis*, and *Listeria monocytogenes*. *Appl Environ Microbiol* 65(9):4276–4279
 37. Al-Haq MI, Seo Y, Oshita S, Kawagoe Y (2002) Disinfection effects of electrolyzed oxidizing water on suppressing fruit rot of pear caused by *Botryosphaeria berengeriana*. *Food Res Int* 35 (7):657–664
 38. Kim C, Hung YC, Brackett RE (2000) Roles of oxidation-reduction potential in electrolyzed oxidizing and chemically modified water for the inactivation of food-related pathogens. *J Food Prot* 63:19–24
 39. Liao LB, Chen WM, Xiao XM (2007) The generation and inactivation mechanism of oxidation-reduction potential of electrolyzed oxidizing water. *J Food Eng* 78(4):1326–1332
 40. Park H, Hung Y-C, Chung D (2004) Effects of chlorine and pH on efficacy of electrolyzed water for inactivating *Escherichia coli* O157:H7 and *Listeria monocytogenes*. *Int J Food Microbiol* 91 (1):13–18
 41. McKenna SM, Davies KJ (1988) The inhibition of bacterial growth by hypochlorous acid. Possible role in the bactericidal activity of phagocytes. *Biochem J* 254(3):685–692
 42. Barrette WC Jr, Hannum DM, Wheeler WD, Hurst JK (1989) General mechanism for the bacterial toxicity of hypochlorous acid: abolition of ATP production. *Biochemistry* 28(23):9172–9178
 43. Yonemori S, Takimoto Y, Min KH, Jitsugiri Y, Simohira T, Miyake H (1997) Analysis of hydroxyl radical generated in electrolyzed strong acid aqueous solution by electron spin resonance spectroscopy. *Nippon Kagaku Kaishi* 7:497–501
 44. Suzuki T, Noro T, Kawamura Y, Fukunaga K, Watanabe M, Ohta M, Sugiue H, Sato Y, Kohno M, Hotta K (2002) Decontamination of aflatoxin-forming fungus and elimination of aflatoxin mutagenicity with electrolyzed NaCl anode solution. *J Agric Food Chem* 50(3):633–641
 45. Comninellis C (1994) Electrocatalysis in the electrochemical conversion/combustion of organic pollutants for waste water treatment. *Electrochim Acta* 39(11–12):1857–1862
 46. Xiong K, Liu HJ, Liu R, Li LT (2010) Differences in fungicidal efficiency against *Aspergillus flavus* for neutralized and acidic electrolyzed oxidizing waters. *Int J Food Microbiol* 137(1):67–75
 47. Martínez-De Jesús FR, Ramos-De la Medina A, Remes-Troche JM, Armstrong DG, Wu SC, Lázaro Martínez JL, Beneit-Montesinos JV (2007) Efficacy and safety of neutral pH superoxidised solution in severe diabetic foot infections. *Int Wound J* 4(4):353–362
 48. Selkon JB, Babb JR, Morris R (1999) Evaluation of the antimicrobial activity of a new super-oxidized water, Sterilox®, for the disinfection of endoscopes. *J Hosp Infect* 41(1):59–70
 49. Park E-J, Alexander E, Taylor GA, Costa R, Kang D-H (2009) The decontaminative effects of acidic electrolyzed water for *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* on green onions and tomatoes with differing organic demands. *Food Microbiol* 26(4):386–390
 50. Oomori T, Oka T, Inuta T, Arata Y (2000) The efficiency of disinfection of acidic electrolyzed water in the presence of organic materials. *Anal Sci* 16(4):365–369
 51. Tanaka N, Fujisawa T, Daimon T, Fujiwara K, Yamamoto M, Abe T (1999) The effect of electrolyzed strong acid aqueous solution on hemodialysis equipment. *Artif Organs* 23(12):1055–1062
 52. Gutierrez AA (2006) The science behind stable, super-oxidized water. *Wounds* 18(Suppl 1):7–10
 53. Shimada K, Ito K, Murai S (2000) A comparison of the bactericidal effects and cytotoxic activity of three types of oxidizing water, prepared by electrolysis, as chemical dental plaque control agents. *Int J Antimicrob Agents* 15(1):49–53
 54. Vorobjeva NV, Vorobjeva LI, Khodjaev EY (2004) The bactericidal effects of electrolyzed oxidizing water on bacterial strains involved in hospital infections. *Artif Organs* 28(6):590–592
 55. Horiba N, Hiratsuka K, Onoe T, Yoshida T, Suzuki K, Matsumoto T, Nakamura H (1999) Bactericidal effect of electrolyzed neutral water on bacteria isolated from infected root canals. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 87(1):83–87
 56. Kim C, Hung Y-C, Brackett RE (2000) Efficacy of electrolyzed oxidizing (EO) and chemically modified water on different types of foodborne pathogens. *Int J Food Microbiol* 61(2–3):199–207
 57. Tanaka H, Hirakata Y, Kaku M, Yoshida R, Takemura H, Mizukane R, Ishida K, Tomono K, Koga H, Kohno S, Kamihira S (1996) Antimicrobial activity of superoxidized water. *J Hosp Infect* 34(1):43–49
 58. Park H, Hung Y-C, Brackett RE (2002) Antimicrobial effect of electrolyzed water for inactivating *Campylobacter jejuni* during poultry washing. *Int J Food Microbiol* 72(1–2):77–83
 59. Issa-Zacharia A, Kamitani Y, Tiisekwa A, Morita K, Iwasaki K (2010) In vitro inactivation of *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* spp. using slightly acidic electrolyzed water. *J Biosci Bioeng* 110(3):308–313
 60. Zeng X, Tang W, Ye G, Ouyang T, Tian L, Ni Y, Li P (2010) Studies on disinfection mechanism of electrolyzed oxidizing water on *E. coli* and *Staphylococcus aureus*. *J Food Sci* 75(5):M253–M260
 61. Landa-Solis C, González-Espinosa D, Guzmán-Arriaga B, Snyder M, Reyes-Terán G, Torres K, Gutierrez-AA (2005) Microcyn™: a novel super-oxidized water with neutral pH and disinfectant activity. *J Hosp Infect* 61(4):291–299
 62. Shetty N, Srinivasan S, Holton J, Ridgway GL (1999) Evaluation of microbicidal activity of a new disinfectant: Sterilox® 2500 against *Clostridium difficile* spores, *Helicobacter pylori*, vancomycin resistant *Enterococcus* species, *Candida albicans* and several *Mycobacterium* species. *J Hosp Infect* 41(2):101–105
 63. Masuda T, Oikawa K, Oikawa H, Sato S, Sato K, Kano A (1995) Endoscope disinfection with acid electrolyzed water. *Dig Endosc* 7(1):61–64
 64. Baltch AL, Smith RP, Franke MA, Ritz WJ, Michelsen P, Bopp LH, Singh JK (2000) Microbicidal activity of MDI-P against *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Legionella pneumophila*. *Am J Infect Control* 28(3):251–257
 65. Fabrizio KA, Cutter CN (2003) Stability of electrolyzed oxidizing water and its efficacy against cell suspensions of *Salmonella typhimurium* and *Listeria monocytogenes*. *J Food Prot* 66:1379–1384
 66. Kiura H, Sano K, Morimatsu S, Nakano T, Morita C, Yamaguchi M, Maeda T, Katsuoaka Y (2002) Bactericidal activity of electrolyzed acid water from solution containing sodium chloride at low concentration, in comparison with that at high concentration. *J Microbiol Methods* 49(3):285–293
 67. Fenner DC, Bürge B, Kayser HP, Wittenbrink MM (2006) The anti-microbial activity of electrolysed oxidizing water against microorganisms relevant in veterinary medicine. *J Vet Med B Infect Dis Vet Public Health* 53(3):133–137

68. Robinson GM, Lee SWH, Greenman J, Salisbury VC, Reynolds DM (2010) Evaluation of the efficacy of electrochemically activated solutions against nosocomial pathogens and bacterial endospores. *Lett Appl Microbiol* 50(3):289–294
69. Park H, Hung YC, Kim C (2002) Effectiveness of electrolyzed water as a sanitizer for treating different surfaces. *J Food Prot* 65:1276–1280
70. Buck JW, van Iersel MW, Oetting RD, Hung Y-C (2002) In vitro fungicidal activity of acidic electrolyzed oxidizing water. *Plant Dis* 86(3):278–281
71. Loshon CA, Melly E, Setlow B, Setlow P (2001) Analysis of the killing of spores of *Bacillus subtilis* by a new disinfectant, Sterilox®. *J Appl Microbiol* 91(6):1051–1058
72. Venczel LV, Arrowood M, Hurd M, Sobsey MD (1997) Inactivation of *Cryptosporidium parvum* oocysts and *Clostridium perfringens* spores by a mixed-oxidant disinfectant and by free chlorine. *Appl Environ Microbiol* 63(4):1598–1601
73. Thorn RMS, Greenman J, Austin AJ (2005) In vitro method to assess the antimicrobial activity and potential efficacy of novel types of wound dressings. *J Appl Microbiol* 99(4):895–901
74. Iwasawa A, Nakamura Y (1993) Antimicrobial activity of aqua oxidized water. *Clin Bacteriol* 20:469–473
75. Tatsumi H, Kuroda H, Takemoto T, Murai T (1994) Bactericidal effects of aqua oxidation water. *Shika Igaku* 57:403–407
76. Fabrizio KA, Sharma RR, Demirci A, Cutter CN (2002) Comparison of electrolyzed oxidizing water with various antimicrobial interventions to reduce *Salmonella* species on poultry. *Poult Sci* 81(10):1598–1605
77. Tapper RC, Smith JR, Cocking C, Beech IB (1998) Atomic force microscopy study of the biocidal effect of super-oxidised water, Sterilox. *Biofilm* 3(1):4
78. Zinkevich V, Beech IB, Tapper R, Bogdarina I (2000) The effect of super-oxidized water on *Escherichia coli*. *J Hosp Infect* 46(2):153–156
79. Cloete TE, Thantsha MS, Maluleke MR, Kirkpatrick R (2009) The antimicrobial mechanism of electrochemically activated water against *Pseudomonas aeruginosa* and *Escherichia coli* as determined by SDS-PAGE analysis. *J Appl Microbiol* 107(2):379–384
80. Nicholson WL, Munakata N, Horneck G, Melosh HJ, Setlow P (2000) Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiol Mol Biol Rev* 64(3):548–572
81. Setlow P (2000) Resistance of bacterial spores. In: Stortz G, Hengge-Aronis R (eds) *Bacterial stress responses*. ASM Press, Washington, pp 217–230
82. Rogers JV, Ducatte GR, Choi YW, Early PC (2006) A preliminary assessment of *Bacillus anthracis* spore inactivation using an electrochemically activated solution (ECASOL™). *Lett Appl Microbiol* 43(5):482–488
83. Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. *Science* 284(5418):1318–1322
84. Mah T-FC, O'Toole GA (2001) Mechanisms of biofilm resistance to antimicrobial agents. *Trends Microbiol* 9(1):34–39
85. Jefferson KK (2004) What drives bacteria to produce a biofilm? *FEMS Microbiol Lett* 236(2):163–173
86. Thantsha MS, Cloete TE (2006) The effect of sodium chloride and sodium bicarbonate derived anolytes, and anolyte–catholyte combination on biofilms. *Water SA* 32(2):237–242
87. Venkitanarayanan KS, Ezeike GO, Hung YC, Doyle MP (1999) Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on plastic kitchen cutting boards by electrolyzed oxidizing water. *J Food Prot* 62:857–860
88. Hata G, Uemura M, Weine FS, Toda T (1996) Removal of smear layer in the root canal using oxidative potential water. *J Endod* 22(12):643–645
89. Marais JT, Brözel VS (1999) Electro-chemically activated water in dental unit water lines. *Br Dent J* 187(3):154–158
90. Lisle JT, Rose JB (1995) *Cryptosporidium* contamination of water in the USA and UK: a mini-review. *Aqua* 44:103–117
91. Suzuki T, Itakura J, Watanabe M, Ohta M, Sato Y, Yamaya Y (2002) Inactivation of staphylococcal enterotoxin-A with an electrolyzed anodic solution. *J Agric Food Chem* 50(1):230–234
92. Tagawa M, Yamaguchi T, Yokosuka O, Matsutani S, Maeda T, Saisho H (2000) Inactivation of a hepatitis virus by electrolysed acid water. *J Antimicrob Chemother* 46(3):363–368
93. Morita C, Sano K, Morimatsu S, Kiura H, Goto T, Kohno T, Hong WU, Miyoshi H, Iwasawa A, Nakamura Y, Tagawa M, Yokosuka O, Saisho H, Maeda T, Katsuoka Y (2000) Disinfection potential of electrolyzed solutions containing sodium chloride at low concentrations. *J Virol Methods* 85(1–2):163–174
94. Park GW, Boston DM, Kase JA, Sampson MN, Sobsey MD (2007) Evaluation of liquid- and fog-based application of Sterilox hypochlorous acid solution for surface inactivation of human norovirus. *Appl Environ Microbiol* 73(14):4463–4468
95. Kitano J, Kohno T, Sano K, Morita C, Yamaguchi M, Maeda T, Tanigawa N (2003) A novel electrolyzed sodium chloride solution for the disinfection of dried HIV-1. *Bull Osaka Med Coll* 48:29–36
96. Shimizu Y, Hurusawa T (1992) Antiviral, antibacterial, and antifungal actions of electrolyzed oxidizing water through electrolysis. *Dental J* 37:1055–1062
97. Kakimoto K, Hayashi K, Nakano T, Sano K, Shimokawa T, Nakai M (1997) Effects of electrolytic products of sodium chloride on HIV-1 infectivity and HBs immunoreactivity. *Environ Infect* 12:1–4
98. Imatanaka N, Yamasaki K, Shiraishi K, Kaziwara T, Nakayama T, Arai K (1994) Single dose and 28-day repeated dose oral toxicity studies of superoxidized water in rats. *Pharmacometrics* 48(3):159–171
99. Morita C, Nishida T, Ito K (2011) Biological toxicity of acid electrolyzed functional water: effect of oral administration on mouse digestive tract and changes in body weight. *Arch Oral Biol* 56(4):359–366
100. Ohtaki Y (1997) The safety of ESAAS. In: Kenkyukai W (ed) *The basic knowledge of ESAAS*. Ohmsha, Tokyo, pp 73–89
101. Shiba A, Shiba K (1997) *A handbook for electrolyzed acidic water*. Igakujohosya, Tokyo
102. Tsuji S, Kawano S, Oshita M, Ohmae A, Shinomura Y, Miyazaki Y, Hiraoka S, Matsuzawa Y, Kamada T, Hori M, Maeda T (1999) Endoscope disinfection using acidic electrolytic water. *Endoscopy* 31(7):528–535
103. Marais JT (2002) Biocompatibility of electrochemically aqueous activated solutions: an animal study. *SADJ* 57(1):12–16
104. Iwasawa A, Nakamura Y (2003) Cytotoxic effect of antiseptics: comparison in vitro. In vivo examination of strong acidic electrolyzed water, povidone–iodine, chlorhexidine and benzalkonium chloride. *Kansenshogaku Zasshi* 77(5):316–322
105. Shimmura S, Matsumoto K, Yaguchi H, Okuda T, Miyajima S, Negi A, Shimazaki J, Tsubot K (2000) Acidic electrolysed water in the disinfection of the ocular surface. *Exp Eye Res* 70(1):1–6
106. Katsuragi H, Suzuki A, Nagaso K, Okamura K, Saito K (1996) Cytotoxicity of aqua-oxidized water. *Jpn J Oral Biol* 38:57–64
107. Serper A, Calt S, Dogan AL, Guc D, Ozçelik B, Kuraner T (2001) Comparison of the cytotoxic effects and smear layer removing capacity of oxidative potential water, NaOCl and EDTA. *J Oral Sci* 43(4):233–238
108. Ayeabah B, Hung Y-C (2005) Electrolyzed water and its corrosiveness on various surface materials commonly found in food processing facilities. *J Food Process Eng* 28(3):247–264
109. Sekiya S, Ohmori K, Harii K (1997) Treatment of infectious skin defects or ulcers with electrolyzed strong acid aqueous solution. *Artif Organs* 21(1):32–38

110. Nakae H, Inaba H (2000) Effectiveness of electrolyzed oxidized water irrigation in a burn-wound infection model. *J Trauma* 49(3):511–514
111. Nakae H, Inaba H (2000) Electrolyzed strong acid aqueous solution irrigation promotes wound healing in a burn wound model. *Artif Organs* 24(7):544–546
112. Altamirano AM (2006) Reducing bacterial infectious complications from burn wounds. *Wounds* 16(S1):17–19
113. Horita Y, Miyazaki M, Noguchi M, Tadokoro M, Taura K, Ozono Y, Kohno S (2000) Healing of Fournier's gangrene of the scrotum in a haemodialysis patient after conservative therapy alone. *Nephrol Dial Transpl* 15(3):419–421
114. Dalla Paola L (2006) Treating diabetic foot ulcers with superoxidized water. *Wounds* 18(S1):14–16
115. Selkon JB, Cherry GW, Wilson JM, Hughes MA (2006) Evaluation of hypochlorous acid washes in the treatment of chronic venous leg ulcers. *J Wound Care* 15(1):33–37
116. Allie D (2006) Super-oxidized dermacyn in lower-extremity wounds. *Wounds* 18(S1):3–6
117. Bongiovanni CM (2006) Superoxidized Water Improves Wound Care Outcomes in Diabetic Patients. *Diabetic Microvascular Complications Today*, May–June, pp. 11–14
118. Hadi SF, Khaliq T, Bilal N, Sikandar I, Saaiq M, Zubair M, Aurangzeb S (2007) Treating infected diabetic wounds with superoxidized water as anti-septic agent: a preliminary experience. *J Coll Physicians Surg Pak* 17:740–743
119. Wolvos TA (2006) Advanced wound care with stable, super-oxidized water. *Wounds* 18(S1):11–13
120. Dalla Paola L, Brocco E, Senesi A, Ninkovic S, Mericvo M, De Vido D (2005) Use of Dermacyn, a new antiseptic agent for the local treatment of diabetic foot ulcers. *J Wound Healing* 2:201
121. Piaggese A, Goretti C, Mazzurco S, Tascini C, Leonildi A, Rizzo L, Tedeschi A, Gemignani G, Menichetti F, Del Prato S (2010) A randomized controlled trial to examine the efficacy and safety of a new super-oxidized solution for the management of wide postsurgical lesions of the diabetic foot. *Int J Low Extrem Wounds* 9(1):10–15
122. Ohno H, Higashidate M, Yokosuka T (2000) Mediastinal irrigation with superoxidized water after open-heart surgery: the safety and pitfalls of cardiovascular surgical application. *Surg Today* 30(11):1055–1056
123. Ichihara T, Fujii G, Eda T, Sasaki M, Ueda Y (2004) The efficacy of function water (electrolyzed strong acid solution) on open heart surgery; postoperative mediastinitis due to methicillin-resistant *Staphylococcus aureus*. *Kyobu Geka* 57(12):1110–1112
124. Desai A, Tam CJ, Bhakta K, Desai T, Sarkar R, Desai NB (2004) The efficacy and tolerability of electrolyzed oxidized water in treating mild to moderate acne. *Cosmet Dermatol* 17(2):93–105
125. Ito K, Nishida T, Murai S (1996) Inhibitory effects of acid water prepared by an electrolysis apparatus on early plaque formation on specimens of dentine. *J Clin Periodontol* 23(5):471–476
126. Solovyeva AM, Dummer PMH (2000) Cleaning effectiveness of root canal irrigation with electrochemically activated anolyte and catholyte solutions: a pilot study. *Int Endod J* 33(6):494–504
127. Marais JT, Williams WP (2001) Antimicrobial effectiveness of electro-chemically activated water as an endodontic irrigation solution. *Int Endod J* 34(3):237–243
128. Hope CK, Garton SG, Wang Q, Burnside G, Farrelly PJ (2010) A direct comparison between extracted tooth and filter-membrane biofilm models of endodontic irrigation using *Enterococcus faecalis*. *Arch Microbiol* 192(9):775–781
129. Hata G, Hayami S, Weine FS, Toda T (2001) Effectiveness of oxidative potential water as a root canal irrigant. *Int Endod J* 34(4):308–317
130. Fraise AP (1999) Choosing disinfectants. *J Hosp Infect* 43(4):255–264
131. Walker JT, Bradshaw DJ, Fulford MR, Marsh PD (2003) Microbiological evaluation of a range of disinfectant products to control mixed-species biofilm contamination in a laboratory model of a dental unit water system. *Appl Environ Microbiol* 69(6):3327–3332
132. Kramer A, Schwebke I, Kampf G (2006) How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis* 6:130
133. Clark J, Barrett SP, Rogers M, Stapleton R (2006) Efficacy of super-oxidized water fogging in environmental decontamination. *J Hosp Infect* 64(4):386–390
134. Prince EL, Muir AVG, Thomas WM, Stollard RJ, Sampson M, Lewis JA (2002) An evaluation of the efficacy of Aqualox for microbiological control of industrial cooling tower systems. *J Hosp Infect* 52(4):243–249
135. Brown CM, Nuorti PJ, Breiman RF, Hathcock AL, Fields BS, Lipman HB, Llewellyn GC, Hofmann J, Cetron M (1999) A community outbreak of Legionnaires' disease linked to hospital cooling towers: an epidemiological method to calculate dose of exposure. *Int J Epidemiol* 28:353–359
136. Yoh M, Akiyama Y, Shimokawa T, Honda T (1994) Flow water hand washing with electrolytic product of sodium chloride. *J Jap Soc Environ Infect* 9:20–23
137. Otoguro K, Suzuki F, Akimaru Y, Iijima H, Yajima Y, Uebaba K (1996) Hand disinfectant activities of two kinds of electrolyzed acid aqueous solutions by globe juice method. *Envir Toxicol Water Quality* 11:117–122
138. Izumi R, Shimaoka M, Nagaoka C, Komaki M, Mizutani A, Yoh M, Honda T, Taenaka N, Yoshiya I (1998) The effectiveness of hand-disinfection by a flow water system using electrolytic products of sodium chloride, compared with a conventional method using alcoholic solution in an intensive care unit. *Crit Care* 2:79–80
139. Sakashita M, Iwasawa A, Nakamura Y (2002) Antimicrobial effects and efficacy on habitually hand-washing of strong acidic electrolyzed water—a comparative study of alcoholic antiseptics and soap and tap water. *Kansenshogaku Zasshi* 76(5):373–377
140. Grunwald L (2010) European legislation: Biocide product authorisation and the revision of the BPD. Society for Applied Microbiology Winter Meeting, Royal Society
141. Rutala W, Weber D (2008) Guideline for disinfection and sterilization in healthcare facilities, 2008. Centers for Disease Control and Prevention (CDC). Healthcare Infection Control Practices Advisory Committee (HIPAC)