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REVIEW

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

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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

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(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

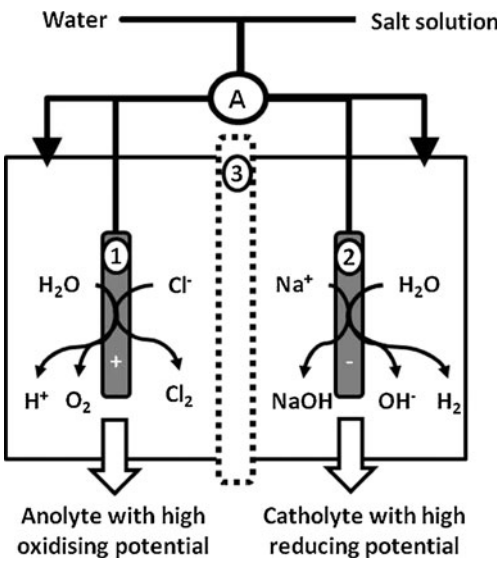

Fig. 1 Prototypical electrochemical cell used for generating electrochemically activated solutions (ECAS), comprising of two electrodes, an anode (1) and a cathode (2), separated by an ion-permeable exchange diaphragm (3). During operation, a salt solution is continuously perfused into both the anodic 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

(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

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

Table 1 (continued)

Target organism	Experimental kill rates (k) of various ECAS[a] ($\log_{10}$ CFUml^{-1} reduction per minute)	
Bacterial spores		
Bacillus anthracis		0.2 [70]
Bacillus atrophaeus	3.7 [68]	0.4–2.0 [52, 61]
Bacillus cereus	1.32–6.98 [54, 56]	
Bacillus subtilis	0.9 [66]	1.0–15.0 [48, 71]
Clostridium difficile	16.3 [68]	2.0 [62]
Clostridium perfringens		0.04 [72]
Streptomyces spp.	+ [28]	+ [28]
Eukaryotes		
Aspergillus spp.	1.48 [46]	5.25 [46]
Candida spp.	3.5 [62]	3.5–16.0 [48, 61, 62, 64]
Cryptosporidium parvum 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 $\log_{10}$ 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 (LD_{50}) 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 putrefacting 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 severalhospital 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 (H_2O 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
• Broad-spectrum antimicrobial activity	• Initial expenditure on generator
• Rapid disinfection time	• Generator servicing and maintenance
• Inexpensive	• Limited shelf life
• Easily accessible raw materials	• Inactivated by organic loading
• On-site or in-situ generation	• Acidic ECAS[a] can be corrosive
• Requires little operator skill	
• Limited toxicity	
• Environmentally compatible	
• Evidence of being anti-inflammatory	

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 antisepsis, and find common usage within healthcare environments.

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