INTRODUCTION

The current pandemic of novel coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is a global crisis (Geller et al., 2012; Hirschmann et al., 2020; Kampf et al., 2020). Currently used disinfectants, including ethanol and hypochlorous acid (HClO), exhibit significant virucidal and microbicidal activities and is compatible with living tissues and the environment. This minireview summarizes recent progress in the development of disinfectants from scallop shell-CaO, focusing especially on studies of clinical and daily use applications. We describe the preparation, basic characteristics, and virucidal and microbicidal activities of scallop shell-CaO disinfectants. Furthermore, their applications in the disinfection of contaminated masks and the treatment of infected wounds are briefly introduced.

Key words: Antiseptic / Calcium oxide / Disinfectant / Scallop shell.

INTRODUCTION

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Calcium oxide (CaO) and calcium hydroxide (Ca(OH)_2) have been widely used for the disinfection of various pathogens. For instance, quicklime and slaked lime produced from limestone are commonly used to disinfect animal houses on a regular basis and during epidemic outbreaks, including that of bird flu (European Lime Association, 2009). Although the major source of CaO and Ca(OH)_2, limestone, is composed mainly of calcium carbonate (CaCO_3), it tends to contain various metal oxides and other impurities (Sdiri et al., 2012; Lai et al., 2020). On the other hand, seashells are known...
CaCO₃ → CO₂ → CaO

FIG. 1. Heat treatment of scallop shells. (a) Chemical reaction. (b) A photograph of the product: scallop shell-CaO in powdered state.

Studies have revealed excellent microbicidal and virucidal activities of heated scallop shells against various pathogenic bacteria (Sawai et al., 2001b), viruses (Thammakarn et al., 2014), bacterial spores (Sawai et al., 2003; Watanabe et al., 2014), fungi (Xing et al., 2013), and biofilms (Kubo et al., 2013; Sawai et al., 2013; Shimamura et al., 2015). Remarkably, the application of heated scallop shells in the disinfection of food products has been investigated due to the low impurity content (Sawai et al., 2001a; Bodur et al., 2010; Cagri-Mehmetoglu, 2011; Kim et al., 2011; Hiruma et al., 2020). The microbicidal and virucidal activities of heated scallop shells are considered to mainly originate from the alkalinity of hydrated CaO or Ca(OH)₂ (Sawai, 2011). Nevertheless, the bactericidal activity was shown to be substantially higher than that of sodium hydroxide (NaOH) at the same pH, indicating that other factors affect the activity (Sawai et al., 2001b). Reactive oxygen species generated by CaO may play important roles (Sawai et al., 1996).

Their disinfection activity, environmental friendliness, and safety for humans make heated scallop shells promising as raw materials for next-generation disinfectants. This minireview summarizes recent progress in the development of disinfectants from scallop shell-CaO, that is, heated scallop shells with a high CaO content, focusing especially on studies of clinical and daily use applications.

Disinfectants can be prepared from scallop shell-CaO by adding it to water. Scallop shell-CaO aqueous solutions exhibit bactericidal activity at 0.1 g L⁻¹, while higher concentrations provide higher activities mainly due to an increase in pH (Sawai et al., 2001b). Scallop shell-CaO solutions have been shown to exhibit higher bactericidal activities than conventional disinfectants, including hypochlorous acid at pH 6, sodium hypochlorite at pH 8, povidone-iodine (Isodine solution®), and chlorhexidine gluconate (Hibiscrub®), in environments contaminated with organic species (Sato et al., 2019b; Fukuda et al., 2020). CaO has a solubility of ~1 g L⁻¹ in water under ambient conditions; the addition of scallop shell-CaO to water at > ~1 g L⁻¹ results in suspensions that generally have a pH of ~12.5, irrespective of concentration, due to saturation (Fig. 2a). Nevertheless, scallop shell-CaO suspensions have been shown to exhibit higher sporicidal activities at higher concentrations, indicating that solid CaO particles also play an important role (Sawai et al., 2003). It is noted that scallop shell-CaO must contain soluble and insoluble ingredients other than CaO, the effects of which have yet to be fully investigated. The findings clearly indicate that higher concentrations of scallop shell-CaO are desirable for preparing effective disinfectants. However, at high concentrations, scallop shell-CaO suspensions readily plug spray nozzles with precipitates, while conventional disinfectants (e.g., ethanol and hypochlorous acid aqueous solutions) are usually used with spraying for clinical and daily use.

**SCALLOP SHELL-CaO SUSPENSIONS, DISPERSIONS, AND FLOCCULATES**

| Additive  | NaH₂PO₄ | Polyphosphate |
|----------|---------|-------------|
| Additive  | (-)     | (-)         |

FIG. 2. Scallop shell-CaO disinfectants. Photographs of (a) the suspensions, (b) the dispersions with NaH₂PO₄, (c) the flocculates with polyphosphate, (d) and the solutions.
We explored the scallop shell-CaO/water system to overcome the issues associated with precipitation. It was found that the addition of phosphate species, such as sodium dihydrogen phosphate (NaH₂PO₄), to scallop shell-CaO suspensions yielded stable dispersions (Fig. 2b) (Sato et al., 2019c). The dispersions had a pH > 12 and contained nanoparticles with diameters of ~200 nm. Incidentally, the dispersions were found to reduce odor and nitrate and nitrite ion concentrations in water, suggesting the potential for environmental application (Sato et al., 2018, 2019c). Another state of scallop shell-CaO/water mixtures was obtained with sodium polyphosphate (Sato et al., 2019a). The mixtures were composed of a transparent upper layer and turbid lower layer of flocculated scallop shell-CaO particles with polyphosphate (Fig. 2c). In vitro assays using normal bacterial flora revealed microbicidal activities of the dispersions and the flocculates, indicating the potential of the two types of scallop shell-CaO/water mixtures as disinfectants.

**SCALLOP SHELL-CaO SOLUTIONS PREPARED AT LOW TEMPERATURES**

Ordinary scallop shell-CaO solutions and suspensions have pH values up to ~12.5 due to the upper limit of the water solubility of CaO at room temperature. On the other hand, at lower temperatures, CaO has higher solubility (De Mendonça Cavalcante et al., 2010). For example, a commercially available scallop shell-CaO solution, BiSCaO Water (Plus Lab Corp., Kanagawa, Japan), is prepared at < 10 °C and has a pH of ~12.8 (Fig. 2d) (Nakamura et al., 2020). BiSCaO Water lacks large CaO particles, albeit with the presence of nanoparticles (100–200 nm in diameter) to some extent; it can therefore be easily sprayed without plugging spray nozzles.

In general, although scallop shell-CaO/water mixtures are promising as antiseptics, their high alkalinity has been an issue for application to living tissues. In this context, the high pH of scallop shell-CaO solutions prepared at < 10 °C was shown to decrease from ~12.8 to ~10 in 24 h when the production of white precipitates during stirring in open air for 24 h, while the scallop shell-CaO suspensions, dispersions with NaH₂PO₄, and flocculates with polyphosphate maintained their high pH for more than 24 h (Fig. 3a, b) (Ishihara et al., 2020). Energy dispersive X-ray elemental mapping and X-ray diffraction analysis revealed that the precipitates produced in the scallop shell-CaO solutions for the production of CaCO₃ precipitates. Adapted from ref. Ishihara et al., 2020.

The microbicidal and virucidal activity of scallop shell-CaO solutions with pH ~12.8 was investigated against various pathogenic bacteria and viruses (Nakamura et al., 2020). In vitro assays revealed that scallop shell-CaO solutions with pH ~12.8 could eliminate more than 99.9% of *Escherichia coli* (E. coli) strains NBRC 3972 and O-157:H7, *Pseudomonas aeruginosa* (P. aeruginosa), Salmonella, *Staphylococcus aureus* (S. aureus), influenza A (H1N1), and feline calicivirus within 15 min (Tables 1 and 2). Furthermore, it was shown by in vitro assays contained in the suspensions, dispersions, and flocculates seemed to continuously dissolve and compensate for precipitated Ca²⁺ with CO₃²⁻, resulting in maintenance of high pH values. The decrease in pH in air was found to be enhanced on material surfaces. When scallop shell-CaO solutions were sprayed onto various materials, such as plastic plates, steel plates, wood pieces, and paper, the pH rapidly decreased to ~10 within 20 min (Fig. 4a) (Ishihara et al., 2020). This is attributed to the large air–water interface area when sprayed on surfaces, leading to efficient absorption of CO₂. Moreover, when the solutions were sprayed onto hairless rat skin, the pH decreased more rapidly to < 9.5 within 5 min, possibly due to organic species with buffer effects on skin (Fig. 4b). Notably, none of the skin regions exhibited chapping or inflammation after the experiments. Therefore, scallop shell-CaO solutions prepared at low temperatures, albeit with high alkalinity, would be safe as antiseptics for application in living tissues. Potential applications include the disinfection of hands by spraying and of the mouth and throat by gargling with scallop shell-CaO solutions.

![Image](https://example.com/image.png)
using normal bacterial flora that the microbicidal activity of scallop shell-CaO solutions with pH ~12.8 was higher than those of scallop shell-CaO suspensions, ethanol, and povidone-iodine (Fig. 5). Given their compatibility with living tissues and spraying, scallop shell-CaO solutions with high pH are promising antiseptics/disinfectants in terms of environmental hygiene and public health, especially during a disease pandemic.

APPLICATIONS

Potential applications of scallop shell-CaO disinfectants for clinical and daily use have been investigated. For example, scallop shell-CaO solutions were shown to be useful for the reuse of surgical masks (Nakamura et al., 2020; Hiruma et al., 2021). Surgical masks sprayed with scallop shell-CaO solutions exhibited antibacterial effects (Nakamura et al., 2020). Moreover, spraying of scallop shell-CaO solutions on surgical masks contaminated with normal bacterial flora resulted in a significant decrease in the bacteria. Importantly, spraying scallop shell-CaO solutions onto surgical masks hardly changed the nonwoven structure of surgical masks (Hiruma et al., 2021). The filtration efficiency of masks after disinfection by scallop shell-CaO solutions will be investigated. The current pandemic of COVID-19 has caused a shortage of masks around the world. Scallop shell-CaO solutions would contribute to overcoming the spread of infectious diseases by allowing reuse of masks.

Scallop shell-CaO has been explored for the treatment of infected wounds. In general, the use of antibiotics for wound treatment is discouraged because of an increased risk for allergies and potential for drug resistance. Antiseptics and nonantibiotic antimicrobials, such as povidone-iodine and weakly acidic hypochlorous acid solutions, have been shown to be cytotoxic (McCauley et al., 1989; Kinoda et al., 2016). In fact, daily cleaning
of wounds with hypochlorous acid solutions (pH 6.5) decreased the *P. aeruginosa* bioburden in infected wounds but delayed wound healing (Kuwabara et al., 2018). Limited hypochlorous acid treatments using chitin nanofiber sheet-immobilized silver nanoparticles (Nguyen et al., 2014; Ishihara et al., 2015) were suggested to suppress the negative effects of hypochlorous acid on wound repair (Kuwabara et al., 2020). In this context, scallop shell-CaO exhibited excellent effects; when *P. aeruginosa*-infected wounds on hairless rats were treated with scallop shell-CaO suspensions daily for 3 days and covered with chitin nanofiber sheets, the *P. aeruginosa* bioburden was significantly decreased, while wound repair was enhanced (Takayama et al., 2020a).

Ointments containing scallop shell-CaO have been developed for the treatment of infected wounds (Takayama et al., 2020b). The ointments were prepared by mixing scallop shell-CaO powder with Vaseline. Treatment of the infected wounds with scallop shell-CaO ointments daily for 3 days significantly enhanced wound healing by reducing the *P. aeruginosa* bioburden (Fig. 6). Histological examinations showed significantly advanced tissue granulation and capillary formation in wounds. The results indicate that scallop shell-CaO is a promising antiseptic for the treatment of infected wounds.

### TABLE 1. Activity of scallop shell-CaO solutions with pH ~12.8 against pathogenic microbes (Nakamura et al., 2020)

| Microbe          | 0 min       | 1 min       | 5 min       | 15 min      |
|------------------|-------------|-------------|-------------|-------------|
| *E. coli* NBRC 3972 | (6.7 × 10^6) | 3.6 × 10^6  | < 10*       | < 10*       |
| *E. coli* O-157:H7   | (6.9 × 10^6) | 6.4 × 10^5  | 4.6 × 10^2  | < 10*       |
| *P. aeruginosa*    | (2.7 × 10^5) | 1.4 × 10^4  | < 10*       | < 10*       |
| *Salmonella*       | (7.1 × 10^5) | 2.1 × 10^4  | < 10*       | < 10*       |
| *S. aureus*        | (3.4 × 10^5) | 1.1 × 10^3  | 1.1 × 10^3  | < 10*       |

The values are the number of colony-forming units (CFU). CFU values at 0 min were estimated using samples without scallop shell-CaO treatment (i.e., control samples). < 10* denotes not detected.

### TABLE 2. Activity of scallop shell-CaO solutions with pH ~12.8 against an enveloped virus and a nonenveloped virus (Nakamura et al., 2020)

| Virus            | 0 min   | 1 min   | 5 min   | 15 min  |
|------------------|---------|---------|---------|---------|
| Feline calicivirus | (6.7)   | < 1.5*  | < 1.5*  | < 1.5*  |
| Influenza A (H1N1) | (6.0)   | 1.7     | < 1.5*  | < 1.5*  |

The values are the fifty-percent tissue culture infectious dose (TCID_{50}/mL) values. TCID_{50}/mL values at 0 min were estimated using samples without scallop shell-CaO treatment (i.e., control samples). < 1.5* denotes not detected.

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**FIG. 6**

**FIG. 6.** Effects of scallop shell-CaO ointments on *P. aeruginosa*-infected wounds in hairless rats. Adapted from ref. Takayama et al., 2020b.
CONCLUSION

Recent studies have revealed that scallop shell-CaO shows great promise as a next-generation disinfectant. The advantages of scallop shell-CaO include its high and broad-spectrum microbicidal and virucidal activities, compatibility with living tissues, and environmental compatibility; that is, it is obtained from natural sources and produces no harmful byproducts. Further application-oriented research will reveal the potential of scallop shell-CaO disinfectants for practical application. Meanwhile, mechanistic studies into the compatibility with living tissues as well as the microbicidal and virucidal activities would still be required for the reliable use of scallop shell-CaO as disinfectants, especially as antiseptics.

The COVID-19 outbreak has highlighted the importance of antiseptics and disinfectants, the usage of which has grown significantly all over the world. In today’s globalized world, a pandemic could occur at any time. We expect that scallop shell-CaO, with its excellent characteristics, can contribute to preventing the spread of infectious diseases.

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