The promise of low-intensity ultrasound: A review on sonosensitizers and sonocatalysts by ultrasonic activation for bacterial killing

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Abstract

Antimicrobial resistance has become one of the main public health issues in modern society. Ultrasonic antimicrobial treatment (UAT) is expected to solve the problem of antimicrobial resistance since ultrasonic treatment does not cause drug resistance during inactivation. However, the ultrasonic application is hindered due to the high energy cost. To cast more lights on the ultrasound in tandem with catalysts as a superior strategy for bacterial inactivation, the present review focuses on the UAT with the assistant of continuous development of organic sonosensitizer and inorganic sonocatalyst. With the application of these nanomaterials, the ultrasonic parameters changed from low-frequency and high-power ultrasound to high-frequency and low-power ultrasound. The review also presents the composition of sonosensitizers/sonocatalysts including organic and inorganic nanoparticles and discusses the ultrasonic activation mechanisms triggered by these catalysts. Based on the synergistic effect of ultrasound and catalysts, we discuss the importance of extracellular oxidation and intracellular oxidation in the process of bacterial inactivation. Overall, UAT combined with catalysts appears to be an effective treatment strategy that can be successfully applied in the field of medicine, environmental treatment, and food industry.

1. Introduction

Bacterial resistance occurs when bacterial strains survive from bacterial killing agents through evolution, which becomes one of the major public health problems in modern society [1]. In recent decades, with the development of novel antibiotics in the medical market, more resistant bacterial species appear which may bring the antibacterial therapy back to the pre-antibiotic era [2]. Drug-resistant infections (DRIs) are estimated to cause 50,000 deaths each year in Europe and the United States [3]. By 2050, DRIs may cause 10 million deaths worldwide every year [4]. Besides, it cannot be ignored that resistant bacteria also appear frequently in the process of environmental treatment [5]. In this regard, actions should be taken to avoid this increasingly serious global healthcare crisis from bacterial resistance.

Unlike chemical or drug treatment on bacterial cells, physical treatment such as ultrasound and ultraviolet (UV) does not produce drug resistance, since these physical treatments do not rely on chemical reagents. However, UV inactivation is easily influenced by several factors, such as water quality, light scattering and absorption, cell shading, and organic fouling in UV lamps [6]. On the other hand, ultrasonic inactivation could conquer the above-mentioned limitations, due to its strong penetrating ability. Therefore, ultrasonic inactivation itself, or combined with UV irradiation, could be helpful in the fight against bacterial resistance [7].

Both physical and chemical effects from ultrasonic cavitation count for bacterial inactivation. During ultrasonic irradiation, collapsed microbubbles in water generate extremely high local temperatures and pressures in the critical region of ultrasound [8]. Moreover, mechanical effects, such as shock waves, shear forces, and micro-jets, lead to mechanical destruction and lysis of bacterial cell membranes [9-11]. However, the energy consumption by powerful ultrasound is highly concerned which limits the further application of ultrasonic inactivation of bacterial cells in water.

With the increase of ultrasonic frequency, the mechanical effect will gradually decrease and the chemical effect will gradually increase [12,13]. The collapse of the microbubbles produces H$_2$O$_2$ and reactive oxygen species (ROS), including hydroxyl radicals (•OH), hydroperoxyl radicals (•HO$_2$), and O$_2$, promoting the oxidation reaction [14-16]. •OH, •HO$_2$,
solids to increase the cavitation nucleus and lower the cavitation threshold or can have catalytic action in terms of degrading oxides and generating ROS [26]. It is worth noting that even though the catalytical additives, such as FeSO₄, can quickly degrade oxides, generate ROS, and enhance the inactivation efficiency of acoustic cavitation, the hydrogen peroxide produced by acoustic cavitation is insufficient for bacterial killing [26]. Alternatively, nano-sonosensitizers can not only lower the cavitation threshold as cavitation nucleus [27,28], increase the yield of ROS in the targeted area [29] but also further increase the ROS produced by acoustically cavitation through Fenton reactions [30]. Overall, nano-sonosensitizers can be added into the ultrasonic system as inert solids with catalytic action to enhance the antibacterial effect of cavitation. However, nano-sonosensitizers require a complicated process to be fabricated, and some nano-sonosensitizers are unstable and cytotoxic.

Ultrasound has been applied to microbial inactivation for many years. With the continuous development of nano-sonosensitizers and sonocatalysts, ultrasound/catalyst inactivation strategies have been reported since 2004 [31]. Relying on the library of web of science, we searched the relevant papers on ultrasonic / sonosensitizer for bacterial inactivation. To visually exploit the effect of sonocatalysts on ultrasonic inactivation, Fig. 1 was produced. Sole ultrasonic inactivation is mainly reported from environment treatment and food hygiene, while medical treatment is relatively rare. Meanwhile, the ultrasonic parameters in these works are usually low-frequency (<100 kHz) high-power (>3W/cm²) (Fig. 1a). With the addition of sonosensitizers and sonocatalysts, the ultrasonic parameters applied for UAT could be changed to high-frequency (>100 kHz) and low-power (<3W/cm²) (Fig. 1a). The reduction of energy consumption is beneficial to promote the practical application of ultrasonic technologies. With the reduction of requirements for ultrasonic energy consumption, we hope that ultrasonic inactivation will also be well developed in the field of environment and food.

With the assistance of catalysts, ultrasound can inactivate Gram-positive and Gram-negative bacteria including Staphylococcus aureus (S. aureus), Methicillin-resistant S. aureus (MRSA), Aggregatibacter actinomycetemcomitans, Listeria innocua, Bacillus cereus, Escherichia coli (E. coli), Extended-spectrum β-lactamase (ESBL)-producing E. coli, Porphyromonas gingivalis (P. gingivalis), Acinetobacter baumannii, Pseudomonas aeruginosa, and Legionella pneumophila (Fig. 1b). Some of these bacteria are very difficult to be killed and prone to develop resistance. One of the best-known drug-resistant S. aureus is one major cause of hospital infections worldwide [32,33]. Disease-associated serotypes such as E. coli O157:H7, O121, and O104:H4 are capable of producing lethal toxins which have been found in water and soil [34-36]. In fact, due to the excessive use of antibiotics, many resistant bacteria have been found in water and soil. With the development of ultrasound / sonocatalyst, resistant bacteria in the environment are expected to be effectively controlled.

Some novel works reported that ultrasound could be used by the combination with catalyst and oxidant to kill bacterial cells [37]. Most studies using organic sonosensitizers tend to explore the inactivation path of bacteria rather than the number and types of free radicals from the aspect of sonochemistry (Fig. 1c). A typical example is the study of Xin et al. [38], maltolhexose-modified cholesterol and bacterial reactive lipid composition was used to establish a smart nanoliposome platform. This catalyst can specifically target the bacterial infection sites by activating bacterial specific maltose dextrin transport pathway. When different kinds of inorganic catalysts are applied, the types of free radicals become diverse. In addition to singlet oxygen (O₂•), which was generated using organic sonosensitizers, other free radicals such as hydroxyl radical (•OH), superoxide radicals (O₂•), and H₂O₂ were produced using ultrasound/inorganic sonocatalysts. These ROS have superior oxidation performance for bacterial inactivation. Interestingly, when organic sonosensitizers are combined with inorganic sonocatalysts, the main free radical produced is reported as O₂• and •OH. It is worth further study whether the organic/inorganic composite can improve the bacterial inactivation efficiency by producing a variety of

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\begin{align*}
\text{H}_2\text{O} + \text{Ultrasonication} & \rightarrow \text{•OH} + \text{•H} \\
\text{•H} + \text{O}_2 & \rightarrow \text{HO}_2 \\
\text{•OH} + \text{•OH} & \rightarrow \text{H}_2\text{O}_2 + \text{O} \\
\text{•O} + \text{O}_2 & \rightarrow \text{O}_2 \\
\text{•OH} + \text{•H} & \rightarrow \text{H}_2\text{O} \\
\text{HO}_2 + \text{•H} & \rightarrow \text{H}_2\text{O}_2 \\
2\text{H}_2\text{O}_2 & \rightarrow 2\text{H}_2\text{O} + \text{O}_2
\end{align*}
\]

ROS are generated by the following reactions during US irradiation in water (reactions (1–4)) [16,17]. These oxidation reactions can damage biocompounds such as DNA, RNA, proteins, and lipids membranes [18,19], destroy the function and structure of bacteria, and finally induce cell death.
free radicals in the ultrasonic field. In this regard, in the aspect of bacterial killing, we will discuss the ultrasonic activation mechanism of organic sonosensitizers and inorganic sonocatalysts, separately. We will then analyze the activation mechanism of the composite of organic/inorganic catalysts. It is hoped to help the design of ultrasonic inactivation catalysts.

3. Sonosensitizers/sonocatalysts for ultrasonic inactivation of bacterial cells

The cavitation effect refers to the process of rapid expansion, compression, and collapse of microbubbles in the liquid when sound pressure changes. It is one of the most important ultrasonic biological effects. According to different ultrasound parameters and organizational microenvironment, cavitation effects are divided into inertial cavitation and non-inertial cavitation. Inertial cavitation is closely related to ROS generation. Inertial cavitation bubbles absorb a large amount of sound energy, and the vibration of the bubbles causes violent collapse, generating high temperature (up to 10,000 K) and high pressure (81 MPa), thereby releasing a large amount of energy. So inertial cavitation can induce hydrothermal dissociation to generate $\cdot{\text{OH}}$ and $\cdot{\text{O}}_2^-$ (reaction 8) [24,43],

$$\text{O}_2 + e^- \rightarrow \cdot{\text{O}}_2^-$$

the holes in the valence band may react with molecules in water to form $\cdot{\text{OH}}$ (reaction 9) [44],

$$\text{H}_2\text{O} + h^+ \rightarrow \cdot{\text{OH}} + \text{H}^+$$

at the same time, part of $\cdot{\text{O}}_2^-$ can also be reduced to $\cdot{\text{OH}}$ and $\text{H}_2\text{O}_2$ through electron induction (reactions (10,11)) [45,46].

$$\cdot{\text{O}}_2^- + \text{H}_2\text{O}_2 \rightarrow \cdot{\text{OH}} + \cdot{\text{OH}}^- + \text{O}_2$$

$$2\cdot{\text{O}}_2^- + 2\text{H}^+ \rightarrow \text{O}_2 + \text{H}_2\text{O}_2$$

In this work, the sonosensitizers/sonocatalysts for ultrasonic inactivation are divided into organic sonosensitizers and inorganic sonocatalysts. Organic sonosensitizers are often derived from sonodynamic therapy for tumor treatment [47]. Inorganic catalysts, due to their semiconductor properties, show a variety of free radical generation mechanisms and good inactivation performance [48].

3.1. Organic sonosensitizers

Organic sonosensitizers used in UAT research mainly include porphyrin or its derivatives, xanthone, and the other organic sonosensitizers like curcumin (Cur) and hypocrellin B (Table 1). Porphyrin or
Table 1

Review of ultrasonic inactivation using sonosensitizers and sonocatalysts reported in the literature.

| Types of sonosensitizers | Names of the sonosensitizers | Types of ROS | Parameters of ultrasound | Microorganism | Processing time | Highest inactivation efficiency\(b\) | Ref. |
|--------------------------|-------------------------------|--------------|--------------------------|---------------|----------------|------------------------------------|------|
| Porphyrin organic sonosensitizer | Hematoporphyrin monomethyl ether (HMME) | Not mentioned | 1 MHz/100 Hz PRF 6 W/cm\(^2\) 30% cycle | Staphylococcus aureus (S. aureus) (G\(+)\) | 30 min | 95% | [51] |
| HMME | Not mentioned | 1 MHz/3 W/cm\(^2\) | Porphromonas gingivalis (P. gingivalis) (G-) | 10 min | 99.997% | [52] |
| Fe@ upconversion nanoparticles (UCNP)-HMME | Not mentioned | 1 MHz/2 W/cm\(^2\) | Methicillin-resistant S. aureus (MRSA) (G\(+\)) | 10 min | 70% | [65] |
| Polymer-peptide-porphyrin conjugate (PPPC) | Not mentioned | 1 MHz/1.5 W/cm\(^2\) | Extended-spectrum \(\beta\)-lactamase (ESBL)-producing Escherichia coli (E. coli) (G-) | 60% | [63] |
| Pd @ Pt-T790 | Not mentioned | 1 MHz/0.97 W/cm\(^2\) 50% cycle | MRSA (G\(+\)) | 8 min | 100% | [29] |
| Xanthones organic sonosensitizer | Rose Bengal (RB) | Not mentioned | 1 MHz/0.84 W/cm\(^2\) 50% cycle | S. aureus (G\(+\)) | 1 h | 99.999% | [55] |
| RB | Not mentioned | 1 MHz/2.5 W/cm\(^2\) | Candida albicans (C. albicans) | 5 min | 100% | [66] |
| RB-antimicrobial peptide conjugate | Not mentioned | 1 MHz/2 W/cm\(^2\) 50% cycle | S. aureus (G\(+\)) | 30 min | 99.997% | [56] |
| Erythrosin B | Not mentioned | 20 kHz/0.86–0.90 W/ml | Listeria innocua (G\(+\)) | 10 s | 99.874% | [37] |
| Other organic sonosensitizer | Curcumin (Cur) | Not mentioned | 1 MHz/1.56 W/cm\(^2\) | MRSA (G\(+\)) | 5 min | 99.999% | [60] |
| Cur | Not mentioned | 1 MHz/1.56 W/cm\(^2\) | Bacillus cereus (G\(+\)) | 3 min | 99.999% | [61] |
| Cur | Not mentioned | 1 MHz/3 W/cm\(^2\) | E. coli (G-) | 5 min | 99.000% | [64] |
| Cur | Not mentioned | 1 MHz/2 W/cm\(^2\) 50% cycle | S. aureus (G\(+\)) | 32 min | 99% | [67] |
| Propyl gallate | Not mentioned | 40 kHz/0.092 W/ml | Acinetobacter baumannii (G\(+\)) | 10–45 min | 99.999% | [69] |
| Nano-emodin | Not mentioned | 1 MHz/100 Hz PRF 2 W/cm\(^2\) | Listeria innocua (G\(+\)) | 5–30 min | 99.000% | [70] |
| Photodithazine | \(^1\)O\(_2\) | 1 MHz/2.5 W/cm\(^2\) | C. albicans | 5 min | 100% | [66] |
| Chlorin e6 | Not mentioned | 1 MHz/1.56 W/cm\(^2\) | S. aureus (G\(+\)) | 5 min | 99.999% | [64] |
| Hypocrellin B | Not mentioned | 1 MHz/1.56 W/cm\(^2\) | MRSA (G\(+\)) | 5 min | 99.000% | [62] |
| MLPI8 | \(^1\)O\(_2\) | 1 MHz/0.97 W/cm\(^2\) | MRSA(G\(+\)) | 5 min | 95% | [38] |
| Amphotericin B | Not mentioned | 42 kHz/0.30 W/cm\(^2\) | ESBL-producing E. coli(G\(-\)) | 80% | 99.65% | [70] |
| Inorganic sonocatalysts | TiO\(_2\) | Not mentioned | 25 kHz/50 W | E. coli(G\(-\)) | 60 min | 95.6% | [31] |
| TiO\(_2\) | Not mentioned | 36 kHz/300 W | Legionella pneumophila (G\(-\)) | 30 min | 99.8% | [71] |
| TiO\(_2\) | Not mentioned | 26 kHz/1.5 W/ml | total coliforms (G\(-\)) | 60 min | 99.9% | [72] |
| ZnO nanofluids | \(^1\)O\(_2\)/\(^1\)OH \(_2\) | 20 kHz/90 W/L | E. coli (G\(-\)) | 10 s | 83% | [17] |
| ZnO\(_{\text{ext}}\) | \(^1\)OH/\(^1\)OH | 20 kHz/20 kHz S. aureus (G\(+\)) | Not mentioned | 90%\(b\) | [74] |
| Au@barium titanate | \(^1\)O\(_2\)/\(^1\)OH | 1 MHz/1.5 W/cm\(^2\) 50% cycle | E. coli (G\(-\)) | 4 min | 99.23% | [24] |
| UCNP@mSiO\(_2\)-(RB)-Ag | \(^1\)O\(_2\)/\(^1\)OH | 2 W/cm\(^2\) | MRSA (G\(+\)) | 10 min | 98.94% | [75] |

(continued on next page)
its derivatives is an effective sonosensitizer in sonodynamic therapy with a stable structure, lower toxicity, higher $^1$O$_2$ yield to induce cell apoptosis via the mitochondrial apoptotic pathway [49], and $^1$O$_2$ is generated by energy transfer between the triplet excited state sensitizer and O$_2$ [50]. Zhuang et al. [51] observed hematoxylinoporphyrin monomethyl ether (HMME) as a sonosensitizer for bacterial inactivation. When HMME was combined with ultrasound for inactivating $P$. gingivalis [52], the expression level of ROS in bacterial cells was significantly increased, suggesting that ROS could be the main cause of cell death. Sun et al. [29] bridged an organic sonosensitizer Mesotetra (4-carboxyphenyl) porphyrin with Pd@Pt nanomaterials. When the sonosensitizers were absorbed by cells, Pd@Pt promoted the decomposition of O$_2$ by endogenous H$_2$O$_2$ to increase the production of endogenous ROS. Wang et al. [53] modified porphyrin with the bacterial targeting peptide to improve the inactivation efficiency.

Xanthone compound Rose Bengal (RB) is not toxic [54], and under sonication, it could react with oxygen to produce $^1$O$_2$. When RB was applied as a sonosensitizer to inactivate S. aureus and E. coli, E. coli showed stronger resistance to UAT than that of S. aureus [55]. Since the special outer membrane of E. coli can effectively prevent RB from entering the cell, it reduces the yield of endogenous ROS and inactivation efficiency. To enhance the inactivation efficiency of RB, Costley et al. [56] coupled bacterial targeting peptides with RB to prepare RB-antimicrobial peptide conjugates, which effectively inactivate Pseudo- monas aeruginosa.

Since Cur and hypocrellin B are natural pigments, Cur could be extracted from popular Indian spice turmeric and hypocrellin B could be isolated from the parasitic fungus Hypocrella bambusae. Besides being used in clinical treatment, recent studies have found that they can also be used as sonosensitizers for ultrasonic inactivation [57-59]. Wang et al. [60,61] found that Cur has a good inactivation effect on S. aureus, E. coli, and Bacillus cereus, which does not depend on intracellular DNA damage. In addition, E. coli, as Gram-negative bacteria, has stronger resistance to Cur/ultrasound. It is probably due to its outer membrane effectively preventing Cur from entering the cell. Wang et al. [62] further investigated hypocrellin B combined with ultrasound for the inactivation of methicillin-resistant S. aureus. Interestingly, they found no DNA damage but the destruction of bacterial membranes which lead to the death of the bacteria.

In addition, there are some other organic sonosensitizers such as nano-emodin [63], chlorin e6 [64], chrysanthemum B [62] (table 1) used for ultrasonic inactivation, and they have achieved good bacterial removal effects. However, organic sonosensitizers have poor water solubility, short blood circulation time, and they are relatively unstable in the environment. To overcome the above shortcomings, researchers are developing inorganic nanomaterials as sonocatalysts.

### 3.2. Inorganic sonocatalysts

Inorganic sonosensitizers are featured as stable physical and chemical properties [80]. At present, regulating the physical and chemical structure of sonocatalysts to generate more free radicals under ultrasound irradiation is a hotspot. Traditional inorganic sonocatalysts such as titanium dioxide and gold nanoparticles have been found to effectively assist ultrasonic inactivation.

As a typical sonocatalyst, TiO$_2$ NPs have been applied to microbial inactivation [31,71,72,81,82]. Ultrasound can excite the electrons in TiO$_2$ NPs from the valence band to the conduction band which will form holes and makes some electron-hole pairs migrate to the surface of the nanoparticles and interact with the surrounding H$_2$O or O$_2$ and such interactions produce ROS such as $^1$O$_2$ and $^1$O$_2$ [25,83,84]. However, due to the fast electron-hole recombination speed of TiO$_2$ NPs (50 ± 30 ns), the yield of ROS is not high [85]. Moreover, the agglomeration of NPs prevents the separation of electron-hole pairs from the energy band, resulting in a further decrease in ROS yield [86]. Changing the structure of TiO$_2$ NPs can also change their catalytic activity. Rahman et al. [40] synthesized a non-woven structure of TiO$_2$ NPs (Fig. 2) combining with ultrasound to effectively inactivate E. coli. The production of $^1$OH significantly increased when non-woven TiO$_2$ NPs were activated by ultrasound. The authors suggested that non-woven TiO$_2$ NPs can provide more cavitation bubbles to enhance the cavitation bioeffects.

Due to its non-toxicity, Au NPs are widely used as nanocarriers for drug delivery [87,88]. Au NPs serve as nucleation sites, lower the cavitation threshold and further increase the cavitation rate [89]. Wu et al. [24] synthesized a piezoelectric nanocomposite material, barium titanate (BaTiO$_3$), BTO nanocubes loaded with Au NPs (Au@BTO NPs), resulting in the separation and migration of electron-hole pairs, which in turn increases the yield of ROS ($^1$O$_2$, $^1$O$_2$) for inactivating S. aureus and E. coli. This work suggested that BTO has an excellent electromechanical conversion rate and high-voltage electrical coefficient. Au NPs are chemically reduced to loaded on the surface of BTO, forming a metal/ semiconductor Schottky junction. This may bend the energy band of BTO and promote the mechanical deformation and piezoelectric effect of BTO caused by the low-intensity ultrasonic mechanical wave.

#### 3.3. The combination of organic sonosensitizers and inorganic sonocatalysts

The combination of organic sonosensitizers and inorganic sonocatalysts can overcome the shortcomings of a single material and even endow the catalyst to realize multi-mechanism inactivation. For example, the electron holes produced by titanium oxide will recombine rapidly, resulting in the decrease of free radical production. To conquer this issue, Wang et al. [78] chemically modified TiO$_2$ NPs with organic substances to inactivate S. aureus. The hybrid catalyst can alleviate the agglomeration of TiO$_2$ NPs, promote the separation of electron-hole pairs from the energy band during ultrasonic activation, and thus increase the ROS yield. Zhao et al. [75] designed a new core–shell nanostructure uponconversion nanoparticles@mSiO$_2$(RB)-Ag NPs. In this kind of NPs, RB, as a sonosensitizer, reacts with O$_2$ in water to produce $^1$O$_2$.
after being activated by ultrasound. It is worth noting that Ag has a long-term inhibitory effect on bacteria. Compared with metal nanomaterials, Si NPs show lower cytotoxicity and have proved to be biodegradable [90-92]. Shevchenko et al. [79] synthesized Dextran-coated Si NPs to inactivate *E. coli*, and all bacteria were killed after 10 min ultrasound irradiation. In addition, iron-based nanomaterials have good Fenton catalytic activity, which expedites the decomposition of H$_2$O$_2$ into O$_2$ to provide a reaction substrate for ROS generation [93]. Following this idea, Wang et al. [65] synthesized Fe@UCNP-HMME NPs for the inactivation of MRSA and ESBL-producing *E. coli*.

Sonosensitizers/sonocatalysts are closely related to the ROS production efficiency of the cavitation effect. Organic sonosensitizers often catalyze to produce *OH and ¹O$_2$. Due to the addition of inorganic sonocatalyst, there are more kinds of free radicals. By studying the physical and chemical structure of inorganic nanomaterials, the mechanism of ROS generation using ultrasound and inorganic sonocatalyst could list as below:

The separation and recombination of electrons and holes at the surface of semiconductors could be mediated to promote the generation of ROS under ultrasonic activation [94,95].

Inorganic nanoparticles with oxygen defect structures can also improve the yield of ROS by efficiently adsorbing H$_2$O$_2$ and O$_2$ in the microenvironment as reaction substrates to further raise ROS production [96,97].

It is also possible to promote the production of ROS by Fenton catalysts [30,98,99].

The combination of organic and inorganic sonocatalysts can produce a variety of free radicals.

4. Cellular Oxidation — Antibacterial Mechanism of ROS

The biological effects of ultrasound can be divided into extracellular oxidation and intracellular oxidation, the former damage the cell membrane through lipid peroxidation, and the latter causes gene and protein damage through oxidative stress (Table 2). It is worth exploring which kind of oxidation is more important in the bacterial inactivation process.

**Fig. 2.** Photograph of non-woven TiO$_2$ fabric (a), surface view of non-woven TiO$_2$ fabric by scanning electron microscopy with different magnifications (b-d).

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### Table 2: Mechanisms of UAT in different works.

| Sonosensitizers          | Purpose         | Mechanisms                          | Ref. |
|--------------------------|-----------------|-------------------------------------|------|
| HMME                     | Medical         | Increase in intracellular ROS       | [52] |
| PPPC                     | Medical         | Cell membrane damage                | [53] |
| Fe@UCNP-HMME             | Medical         | Cell membrane damage                | [65] |
| UCNP@SiO$_2$-RB/HMME     | Medical         | Cell membrane damage                | [76] |
| Pd@Pt-T790               | Medical         | Increase in intracellular ROS       | [29] |
| Cur                      | Food            | DNA damage$^a$                       | [61] |
| Cur                      | Medical         | DNA damage$^a$                       | [60] |
| Cur                      | Medical         | Downregulation in virulence genes   | [68] |
| Hypocrellin B            | Medical         | Cell membrane damage                | [67] |
| Hypocrellin B            | Medical         | Cell membrane damage                | [62] |
| Nano-emodin              | Medical         | DNA damage$^a$                       | [63] |
| Propyl gallate           | Food            | Lipid peroxidation                  | [69] |
| Amphotericin B           | Medical         | Increase in intracellular ROS       | [70] |
| MLP18                    | Medical         | Cell membrane damage                | [38] |
| TiO$_2$                  | Medical         | Oxidative stress response           | [71] |
| Non-woven TiO$_2$        | Environment     | Lipid peroxidation                  | [40] |
| Ti-S-TiO$_2$             | Medical         | Cell membrane damage                | [73] |
| TiO$_2$-Sinoporphyrin    | Medical         | Cell membrane damage                | [78] |
| sodium                   |                 | Increase in intracellular ROS       | [17] |
| ZnO nanofluids           | Environmental   | Cell membrane damage                | [74] |
| ZnO$_{ext}$              | Medical         | Cell membrane damage                | [74] |
| Dextran-coated Si         | Medical         | Cell membrane damage                | [79] |
| UCNP@mSiO$_2$(RB)-Ag      | Medical         | Cell membrane damage                | [75] |
| Au@barium titanate       | Medical         | Lipid peroxidation                  | [24] |

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$a$: The authors conducted related research, but got negative results.

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4.1. Extracellular oxidation — cell membrane damage and lipid peroxidation

For extracellular oxidation, the ROS produced by ultrasound or sonosensitizers can oxidize biological cell membranes [53], since ROS could react with phospholipids, enzymes related to the membrane, side chain of membrane receptor-associated polyunsaturated fatty and
nucleic acid to form lipid peroxidation products [100]. As a result, the fluidity and permeability of the cell membrane are changed, ultimately leading to changes in the structure and function of bacteria, and even cell death. This chain reaction is called lipid peroxidation. Moreover, it provides a continuous supply of ROS, and the newly generated ROS leads to changes in the structure and function of bacteria, and even cell death. Therefore, improving the efficiency of inactivation is critical. Rahman et al. [40] studied the effect of non-woven TiO₂ combined with ultrasound on lipid peroxidation of cell membranes and found that ‘•OH produced by the reaction of non-woven TiO₂ significantly increased the lipid peroxidation level of bacteria membranes. Wu et al. [24] found that Au@BTO NPs combining with ultrasound had an excellent antibacterial efficiency against both Gram-negative E. coli and Gram-positive S. aureus, they deduced that the sonodynamic ROS generation induced lipid peroxidation in cytomembrane, which enhanced the permeability of cell membrane and finally led to the intracellular protein leakage and irreversible damage to bacteria. Martins et al. [101] got the same result in cancer cells, they studied the application of zinc phthalocyanine (ZnPc) as a sonosensitizer and discovered that the level of cellular lipid peroxidation increased three times after sonication.

Studies have shown that the outer membrane of the Gram-negative bacteria will also be oxidized and destroyed by ROS. By cutting the glycoside backbone to break the biopolymer, the composition and function of these cells are changed, which causes bacterial death [102,103]. But the highly organized bacterial outer membrane of Gram-negative bacteria may inhibit the absorption of sonosensitizers, resulting in a lower inactivation effect than that of Gram-positive bacteria [38].

4.2. Intracellular oxidation — cellular oxidative stress response

For intracellular oxidation, oxidative stress refers to the imbalance between the oxidation and anti-oxidation effects of biological cells. When the cells tend to be oxidized, the secretion of proteases increases, and a large amount of oxidative intermediate products are generated. Oxidative stress is a negative effect of oxygen free radicals intracellularly, which is closely related to cell apoptosis and death [104]. In the process of sonication, ultrasound and sonosensitizers produce a large amount of ROS, leading to cellular oxidative stress response, which is also one of the main antimicrobial mechanisms.

Studies have indicated that ROS produced in the cells can lead to the oxidation of intracellular proteins [105]. ‘•OH attacks electron-rich sites, such as the double bond chain and main chain on the amino acid side [106,107]. So the specific function of the corresponding protein is inhibited, leading to dysfunction of the microbial cell and eventually death. In addition, ‘•OH produced by microbial cells themselves can also damage intracellular nucleic acids, such as cutting the double helix structure of nucleic acids or modifying nucleic acids with nitrogen bases [108,109]. The normal physiological functions of microbial cells are interfered, causing cell death. Zhang et al. [52] used HMME combining with ultrasound to inactivate P. gingivalis, they found that UAT can increase the intracellular yield of ROS, and cause the death of bacteria. The same phenomenon was observed by Yang et al. [70], they synthesized amphotericin B-loaded nanoparticles combining with ultrasound to effectively inactivate C. albicans by intracellular ROS produced by UAT. Pourhajibagher et al. further clarified that UAT can down-regulate specific genes in cells. They found that the Curcumin-decorated nanophytosomes-mediated ultrasound could reduce the cell viability, metabolic activity, and biofilm growth in A. actinomycetemcomitans by downregulating the expression of rcpA, qseB, and qseC genes [68]. Meanwhile, nano-emodin-mediated ultrasound could significantly downregulate the expression levels of lasI, agrA, and aba as the virulence genes in P. aeruginosa, S. aureus, and A. baumannii, causing the reduction of the formation of bacterial biofilms and the viability of bacteria [63].

To visually explain the importance of extracellular/intracellular oxidation on ultrasonic inactivation in different works, Fig. 3 was produced according to the studies listed in Table 2. When inorganic sonosensitizers are used alone or in combination, Researchers pay more attention to extracellular oxidation (Fig. 3a), only a few studies include both extracellular oxidation and intracellular oxidation. Meanwhile, medical-related studies focus more on the oxidation effect on cells (Fig. 3b).

Both extracellular and intracellular oxidation may play a very important role in the ultrasonic inactivation process. However, there are still disputes. For example, Wang et al. [61] clarified that ultrasound/Cur did not damage the DNA of E. coli, while the outer membrane significantly affects the antimicrobial effect of UAT. Thus, it seems that intracellular and extracellular oxidation does not necessarily exist at the same time.

5. Conclusion

With the development of functional nanomaterials, ultrasonic inactivation technology is gradually combined with nanomaterial technology, which has brought about significant changes in this field. To cast more lights on the ultrasound in tandem with catalysts for bacterial killing, the key findings are summaries as below:

(1) For bacterial inactivation, low-frequency high-power ultrasound is gradually replaced by high-frequency low-power ultrasound.

(2) Organic sonosensitizers show the advantage of low biological toxicity and produce highly active singlet oxygen. However, the manufacturing cost of these organic sonosensitizers is high and its application field is narrowed in medical application.

(3) With the development of inorganic sonocatalysts, the combination of ultrasound and inorganic sonocatalysts can be better used
in the field of environment and food. Particularly, semiconductor catalysts produce free radicals through hole electron separation in the sound field, which is an interesting mechanism and opens the way for the design of unique inorganic catalysts.

(4) Lipid peroxidation and oxidative stress may not exist at the same time during UAT. In different strategies of ultrasonic inactivation, intracellular and extracellular oxidation may work separately. So far, it is unclear how to accurately regulate intracellular and extracellular oxidation.

In summary, oxidation is the key to ultrasonic inactivation using low-intensity ultrasound. Sonocatalysts can promote the application of UAT from the perspective of increasing the yield of ROS and reducing energy consumption, but it is essential to develop high-efficiency nano-sono-sensitizers and/or sonocatalysts to increase the yield of ROS and clarify the relationship between ROS generation and the regulation of intra-cellular and extracellular oxidation. The review could be helpful for the development of a controllable, efficient, and safe ultrasonic antimicrobial technology.

CRediT authorship contribution statement

Gongdao Wang: Writing – original draft, Formal analysis. Wei Wu: Conceptualization, Funding acquisition, Writing – review & editing. Jun-Jie Zhu: Conceptualization, Writing – review & editing. Danhong Peng: Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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