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Review article

Exploring the Effects of Hydrogen Peroxide on Biofilm Development and Antibiotic Susceptibility

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ABSTRACT

Hydrogen peroxide is one of the most important chemical compounds that affect the formation of biofilm of microorganisms and response to antimicrobial agents, and it can be used in war against pathogenic microorganisms in healthcare institutions. The current study aims to highlight the mechanism of the effect of H_2O_2 on biofilm formation, in addition to the possibility of using it to enhance the ability of antimicrobials to reduce the outbreak of infectious disease. In this review article, the impact of hydrogen peroxide on the susceptibility of pathogenic bacteria to antibiotics will be screened through the role of H_2O_2 in reducing biofilm formation. Here, the mechanism of the effect of H_2O_2 on the body of pathogenic bacteria through oxidation stress, eradication of biofilm, destruction of microbial membrane, and inhibition of microbial enzymes. The effect of hydrogen peroxide on biofilm formation depends on the concentration of hydrogen peroxide and the duration of exposure of microorganisms. At some times, low concentrations of H_2O_2 induce biofilm formation, and high concentrations may reduce the biofilm formation (and that may be dependent on the species of microorganism). The effect of H_2O_2 on biofilm formation is through reducing initial attachment as well as interfering with the maturation and maintenance of biofilm. Hydrogen peroxide has an important role in enhancing antibacterial agents, and this may contribute to redrawing the therapeutic strategy in the future. It can be concluded from the present study that H_2O_2 has a role in reducing the outbreak of infectious diseases and helps enhance the antibacterial war, which contributes to enhancing public health.

Keywords: Antibiotics, Bacterial susceptibility, Biofilm, Developing antibacterial agents, Hydrogen peroxide.

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1. INTRODUCTION

Biofilms are complex microbial communities that form and attach to different surfaces encased in a self-produced extracellular matrix [1]. The biofilm is not just found in plumbing but is also in all sorts of natural and industrial settings. They can form on various surfaces and are often found in nutrient-rich environments. In many instances, processes within natural biofilms are analogous to what happens in artificial or industrial setups [2]. Previous studies showed the elimination of bacterial biomass and biofilm by using hydrogen peroxide (H_2O_2) [3]. Most biofilms are found in environments created by humans: inside houses, in industry, and the medical devices that produce the defects [2]. While it is expected that H_2O_2 would effectively eradicate biofilms by penetrating and disrupting the microbial cells within, recent studies indicate that its effects are more nuanced [4]. Research using computational modeling has demonstrated that biofilms can exhibit unexpected behaviors when exposed to hydrogen peroxide. Previous studies showed that a particular concentration of H2O2 may not eradicate the biofilm but increase the thickness of the biofilm [5]. The dead biomass in the biofilm of bacterial culture protects the viable bacterial cells from the effect of H_2O_2 by neutralizing its effect that protects viable cells in the deep area in the biofilm from oxi-

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oxi stress that yields from the H2O2 [6]. This finding creates the changes to using the disinfected agents against the bacterial biomass in the hospitals that lead to re-evaluating the strategies of using the oxidative agents as disincentive agents on the floor of the health institutions.

The relationship between hydrogen peroxide and antibiotic effectiveness is a key aspect of this review article. When present, H_2O_2 can change how well antibiotics work against biofilms. Biofilms consist of bacteria that stick to surfaces, and they can form in human bodies, for instance, on indwelling catheters. There's currently no good way to treat such infections. The using of H_2O_2 and antibiotics together may reflect positively on treating the bacterial infection and ultimately produce good patient health [7].

Several factors interfere with the effect effects of H2O2 on biofilm development which requires investigation when the researchers want to understand biofilms and their susceptibility to antibiotics. In this review, the effect of H2O2 on the dynamics of biofilm formation to build strategies for managing the bacterial infections that are related to biofilm formation. This will help to improve the effectiveness of antibiotics against the bacterial infection associated with biofilm formation.

2. OVERVIEW of BIOFILM

Biofilm is a biomass of microorganisms that communicate together at the biotic or abiotic surfaces. Commonly known as polysaccharides, proteins, and nucleic acids [1]. This matrix maintains structural integrity and protects biofilms, which enables them to exist in a variety of environments, from ecosystems to medical devices [2]. The development of biofilms occurs in several stages: initial attachment of planktonic cells to a surface, irreversible adhesion due to the production of extracellular polymeric substances, growth into three-dimensional structures that are quite complex, and the shedding of cells that can settle in a new region [8]. Infections are particularly difficult to treat because biofilms are refractory to the host immune response as well as antimicrobial agents [9]. The bacteria within the biofilm communicate with one another through the mechanism of bacterial quorum sensing. This phenomenon is a cell-to-cell communication that uses gene expression controlled by the population density of the microorganisms. Such communication also enables synchronized actions such as nutrient acquisition and biofilm development [10]. Biofilms can be useful in cases of bioremediation and wastewater treatment, however, in the field of medicine, they are of great concern due to their role in chronic infections and device-associated infections [11]. The understanding of biofilm development and related resistance mechanisms to antibiotics is highly important in controlling the infections that are related to biofilm formation

2.1. Important of antibiotic Susceptibility

Antibiotic resistance in bacteria that form biofilm strongly has become a focus of attention because biofilm poses specific problems in both clinical and environmental settings. Biofilms are structured communities of bacteria locked in an extracellular matrix and there is often a marked difference in antibiotic susceptibility between biofilms and planktonic free-floating [12] microbes. The majority of bacteria in biofilms acquire higher resistance to most classes of antibiotics. The basis of these resistances includes factors like restricted diffusion of the antimicrobial agents, and differentiation of cells in the biofilm with slow growth rates reason these cells would be less affected by antibiotics and these cells could also produce enzymes able to inactivate the antimicrobial agents. For instance, *Staphylococcus aureus* biofilm-forming strains have been reported to have high levels of resistance to beta-lactams which pose a critical challenge in the clinical context of biofilm-mediated infections [13]. Biofilms are involved in about 65-80 percent of chronic infections and also in device-associated and implant-associated infections. The ability of biofilm-forming bacteria to escape most of the conventional antibiotics treatment makes it easier to deal with the infection however such patients may need to go through black box procedures including explanation of infected devices of the patients [14].

It is important to gain insight into the association between biofilm formation and antimicrobial sensitivity for efficient treatment methods to be adopted. According to research, some specific antibiotics can stimulate the establishment of biofilms under low doses, pointing toward a more complex relationship of biofilms with antibiotics [15]. With this understanding, the development of other novel means that may help to target biofilms or enhance the effectiveness of already available antibiotics can be achieved. The appearance of multi-drug resistance (MDR) and extensively drug-resistant (XDR) biofilm strains creates a burden in the community. Regular evaluation and monitoring of biofilm-forming bacteria for antibiotic resistance is essential to prevent and treat infections appropriately [16]. Indeed, the investigation of antibiotic resistance in biofilm-forming bacteria is relevant for overcoming the threats of persistent infections and creating potential therapeutic solutions. Ultimately, knowing these phenomena will be valuable in improving clinical outcomes and the management of biofilm infectious diseases.

3. MECHANISMS of ACTION of HYDROGEN PEROXIDE

H_2O_2 is an antimicrobial agent that is widely used for its disinfectant equipment, especially medical devices. Its mechanism of action consists of several biochemical processes that contribute to its effectiveness against many microorganisms including bacteria, viruses, and fungi

- **Production of reactive oxygen species (ROS):** H_2O_2 produces reactive oxygen species. Especially hydroxyl radicals are highly reactive and destroy cellular components, lipids, ROS, proteins, nucleic acids, etc. They can oxidize important biomolecules. This leads to cell death. Oxidative damage disrupts cell membranes and impairs vital cellular functions. Ultimately, it leads to bacterial fragmentation and death [17].
- **Membrane Damage:** H_2O_2 penetrates microbial cell walls and membranes, leading to increased permeability. This disruption allows for the leakage of intracellular contents and the loss of essential ions, further compromising cell integrity. The damage to membrane lipids initiates further oxidative stress within the cell [17,18].
- **Inhibition of Enzymatic Activity:** The H2O2 can inhibit the important metabolic enzymes that are involved in bacterial metabolism that happen by changing the enzyme structure and kinetics process through the oxidation process that may reflect on the bacteria metabolism process that is critical in bacterial growth and survival [19].
- **Biofilm Disruption:** Prior research suggests that H_2O_2 can effectively reduce the biofilm biomass of different species of bacteria. It disturbs the extracellular polymeric substances (EPS) which are the biofilm matrix that protects embedded bacteria from exposure to antimicrobial agents. Hydrogen

peroxide has been shown to dramatically reduce biofilm density and viability with relatively short exposure times [18,20].

Hydrogen peroxide as an antimicrobial agent and its antibiotic mechanisms of action emphasize the efficacy of hydrogen peroxide on various microbial species through oxidative damage, disruption to membranes, and biofilm minimization. Recognizing these mechanisms is crucial for maximizing its potential with applications such as in health care where infectious agents must be contained.

4. IMPACT of H_2O_2 on BIOFILM FORMATION

The previous studies on the effect of Hydrogen peroxide have shown two sward edges. In some cases, the H2O2 promotes biofilm formation and in other studies, the H2O2 eradicates the biofilm biomass. That is dependent on the bacteria population microenvironments and the concentration of H2O2 in the environments of bacterial growth [21]. Duan et al (2016) reported that H2O2 promotes *Aggregatibacter actinomycetemcomitans* and *Streptococcus parasanguinis* to form biofilm [21]. Other investigators reported that H2O2 decreases and eradicates the biofilm formation [22]. There are several keys to the effect of H2O2 on the biofilm formation.

- **Anti-Biofilm Activity**: H_2O_2 is effective in eradicating biofilms formed by various bacterial species, including *Escherichia coli*. Research indicates that concentrations of H_2O_2 at or above 6.25% (v/v), when applied at elevated temperatures (≥40 °C) for sufficient exposure times (≥25 min), can significantly reduce biofilm biomass and viability. This suggests that H_2O_2 disrupts the structural integrity of biofilms, leading to cell lysis and death [23].
- **Temperature and Concentration of H2O2**: The effectiveness of H_2O_2 against biofilms is influenced by factors such as concentration, temperature, and contact time. Higher concentrations and temperatures enhance its ability to penetrate the biofilm matrix and exert lethal effects on embedded bacteria [23].
- **Raise of Biofilm Formation**: Exogenous addition of H_2O_2 in low concentration promotes the production of biofilm in certain bacterial isolates, i.e. *Acinetobacter oleivorans*. This happens because the oxidative stress responses enhance the production of extracellular polymeric substances (EPS), which are vital in biofilm development. The other researchers found that H_2O_2 in certain concentrations stimulates genes associated with EPS production, thereby facilitating initial biofilm formation [24].
- **Tolerance Mechanisms**: The biomass of biofilms shows tolerance to H_2O_2 because of the neutralization of the compound by dead biomass of bacteria. This will help viable cells in the biofilm to survive and get rid of the oxidative stress of H_2O_2. Studies have shown that prolonged exposure to sub-lethal concentrations of H_2O_2 leads to increased thickness and resilience of biofilms in particular bacteria species [6].
- **Long-Term Effects on Biofilms**: Treatment with H_2O_2 concentrations (low concentrations) fails to eradicate established biofilms will stimulate bacterial biomass to regrowth after treatment with H2O2. Cyanobacteria biofilms exposed to H_2O_2 demonstrated increased biomass two weeks after treatment, indicating that disinfecting agents might inadvertently promote biofilm resilience if not applied in sufficient concentration [25].

4.1. H_2O_2 inhibits the initial attachment

Hydrogen Peroxide (H_2O_2) has a major effect on the initial attachment of bacteria which is, an important step during biofilm formation. The influence of H_2O_2 on this process could be elucidated by a variety of mechanisms. **Initial Inhibition of Attachment**, hydrogen peroxide inhibits the ability of bacteria to initially attach to the surface via producing various ROS species experienced by bacterial oxidative stress which can break down cell membranes thus hampering the attachment of bacteria. This is especially important in settings where biofilm formation is considered to be detrimental, such as medical devices and water systems [26]. **Concentration-Dependent Effects**, the effect of H2O2 on bacterial adhesion is concentration-dependent. Hydrogen Peroxide at higher concentrations (≥6.25% v/v) has previously demonstrated the ability to eradicate preformed biofilms and inhibit subsequent biofilm development, resulting in more than a 3-log reduction in total biofilm formation [14]. In comparison, lower concentrations may not have adequate antimicrobial activity to prevent attachment [23, 24]. **Oxidative Stress Response:** Exposure H_2O_2 then bacteria activate a stress response mechanism that temporarily inhibits bacterial adhesion to abiotic surfaces which the effect is the first step in biofilm formation. The oxidative stress induces the expression of detoxification and damage repair genes (*arcA*, *mdoG*, and *tus*) in *E. coli* [27], which can change the bacteria's surface properties affecting its adhesion abilities. Exopolysaccharides (EPS) while in other contexts H_2O_2 can inhibit initial attachment and adhesion of bacteria, it has been observed that biofilm formation is promoted following H_2O_2 treatment due to the induction of exopolysaccharides (EPS) production from some species like *A. oleivorans*. This dual role emphasizes the paradoxical nature of H_2O_2 effects when lower concentration can contribute to EPS production under certain conditions and favor later steps of biofilm development [24]. **Competitive Dynamics**: In competitive environments, some species produce H_2O_2 inhibits the growth of non-adapted ones and favors the growth of adapted species instead. The developing biofilm community composition can be shaped by such selective pressures [26]. Therefore, the antimicrobial action and oxidative stress-inducing capability of H_2O_2 play an important role in the meditating effect maintained on bacterial initial attachment and biofilm formation. It mostly has an inhibitory effect on initial attachment at higher concentrations but also increases biofilm formation generally by inducing exopolysaccharides (EPS) production [26]. Log in or register to get access to the full content.

4.2. Interference with Maturation and Maintenance

The impact of H2O2 on the dynamics of biofilm development, maturation, and maintenance is complex. Hydrogen peroxide influences the stability and maintenance of biofilm. The concentration, duration of exposure, and type of microbial community significantly influence the effect of hydrogen peroxide on biofilm development.

4.2.1. Inhibition of Biofilm Maturation

- **H_2O_2 induces oxidative stress:** Microbial cells subjected to elevated levels of hydrogen peroxide and H2O2 will undergo oxidative stress, hence impeding biofilm development. Hydrogen peroxide can impede bacterial proliferation by destroying biological constituents such as membranes and DNA, therefore breaking stable biofilm formations [18]. The prior work demonstrated that H_2O_2 effectively reduces biofilm thickness and the survival of *Staphylococcus epidermidis*, with optimal concentrations ranging from 3% to 5% [18].

- **Disruption of Extracellular Polymeric Substances (EPS)**: H_2O_2 affects the production and integrity of EPS, which are crucial for biofilm structure. The oxidative action can degrade EPS components, leading to a less stable biofilm matrix and facilitating detachment of *Streptococcus mutans* bacterial cells from the biofilm and also killing the bacteria and helping in the maintenance of the patient teeth [28].

4.2.2. Impact on Biofilm Maintenance

- **Catalase Activity**: The presence of catalase-producing bacteria within a biofilm neutralizes H_2O_2, allowing for the survival of deeper layers while protecting them from oxidative damage. This ability to neutralize H_2O_2 contributes to the maintenance of biofilms that were formed by *Pseudomonas aeruginosa* even during continuous exposure to the disinfectant. Studies using computational models have shown that dead biomass within a biofilm can absorb H_2O_2, providing a protective barrier for living cells deeper within the structure [29].
- **Concentration Thresholds**: Research indicates that sustained low concentrations of H_2O_2 may not be effective in completely eradicating biofilms. Instead, treatments need to exceed a critical threshold concentration to effectively disrupt biofilm maintenance mechanisms. Continuous low-dose exposure may allow for adaptive responses that enhance biofilm resilience. The previous studies support that high concentrations of H_2O_2 can eradicate the biofilm formed by *E. coli* [23] while low concentrations of endogenous H_2O_2 increase the EPS produced by *A. oleivorans* and enhance biofilm formation [24].

4.2.3. Adaptive Responses

Non-lethal exposure to H_2O_2 induces a priming response in bacteria, enhancing their survival against subsequent lethal doses. This adaptive response may involve the up-regulation of stress response pathways and increased efficiency in removing H_2O_2 from their environment, ultimately contributing to biofilm resilience [27].
Hydrogen peroxide significantly interferes with both the maturation and maintenance of biofilms through oxidative stress, disruption of EPS integrity, and the influence of microbial adaptive responses. While it can effectively inhibit biofilm development at higher concentrations, its interaction with catalase activity and potential priming effects complicate its use as a disinfectant.

5. H_2O_2 as an ADJUNCT to ANTIBIOTIC THERAPY

In recent years an interest in the application use of H_2O_2 as a potential adjunct to antibiotic therapy, particularly in the context of combating antibiotic-resistant infections and enhancing the efficacy of existing antimicrobial treatments. Its mechanisms of action are dependent on the oxidation damage of the bacterial cells, biofilm disruption, and synergistic effect with other anti-microbial agents [30]. There wide spectrum of applications of using hydrogen peroxide in clinical applications. Recent advancements have shown that H_2O_2 when combined with blue light therapy, can effectively kill MRSA by destabilizing cell membranes and allowing H_2O_2 to penetrate bacterial cells. This combination has been reported to achieve up to 99.9% bacterial reduction [31]. H_2O_2 has been explored in various chronic infection models. For instance, its application in dental unit water systems has shown significant reductions in microbial load, indicating its potential for broader clinical applications [30].
Despite its potential benefits, there are limitations to the use of H_2O_2 as an adjunct therapy. The effectiveness of H_2O_2 can vary based on concentration and exposure duration. High concentrations may be necessary for effective antimicrobial action but could also lead to tissue damage if not carefully managed [23,24]. The presence of catalase-producing bacteria can neutralize H_2O_2, reducing its effectiveness in certain environments, such as within biofilms or in the bloodstream [32]. H_2O_2 may enhance antibiotic efficacy, but there is a concern regarding potential resistance development in bacteria exposed to sub-lethal concentrations over time [27].
Hydrogen peroxide shows promise as an adjunct to antibiotic therapy by disrupting biofilms, enhancing antibiotic penetration, and exerting oxidative stress on bacteria. Its application in treating resistant infections like MRSA highlights its potential clinical utility. However, careful consideration of concentration, exposure time, and microbial characteristics is essential for optimizing its use. Further research is needed to establish standardized protocols for combining H_2O_2 with antibiotics in clinical settings to maximize therapeutic outcomes while minimizing risks.

6.1. Synergistic Effects with Antibiotics

Hydrogen peroxide's synergistic effects when combined with various antibiotics and other antimicrobial agents illustrate its potential as a treatment for bacterial infections caused by high-resistance bacteria. The use of H_2O_2 in combination with these standard treatments can enhance their antibacterial effects in several ways, including by causing oxidative stress, which makes the bacteria much more susceptible to the simultaneously applied antibiotics; at high enough concentrations, H_2O_2 itself can do this as can other standard treatments like chlorhexidine. Feuerstein et al. (2006) directed another line of research that was aimed at discovering the exact nature of the "synergistic" effect that they had previously reported. They found that blue light and H_2O_2 together exhibited over 96% growth inhibition against *Streptococcus mutans* compared to the much lower growth inhibition levels when these agents were applied separately. The investigators hypothesized that the use of blue light to activate H_2O_2 resulted in a quantum leap in the photosensitization potential of the H_2O_2 and was responsible for the production of a much larger quantity of the reactive oxygen species (ROS) that they had previously identified as being responsible for the enhanced antibacterial effect produced by the use of these two agents in concert [33].
The antibacterial potency of the combination of chlorhexidine (CHX) and hydrogen peroxide (H_2O_2) was equivalent to that of sodium hypochlorite (NaOCl). Thus, it could serve as a useful root canal irrigant. The combined antibacterial effect of CHX and H_2O_2 is greater than either agent could accomplish on its own. The H_2O_2 not only penetrated the bacterial cell walls better than NaOCl could, but it also worked with the CHX to kill off the resistant cells that either agent wouldn't have been able to vanquish alone [34]. Alkawareek et al. (2019) found that silver nanoparticles also work synergistically with H_2O_2 to kill off

bacteria [35]. This synergy came from a Fenton-like reaction where AgNP and H_2O_2 interacted. They produced highly reactive hydroxyl radicals [35]. This was good; these radicals enhanced the antibacterial activity we were studying. There was a downside, though. If not managed properly, that much reactivity could lead to toxicity. So, we managed it. We moderated the interaction of H_2O_2 with gluconic acid in honey. Then we hit the target. We got enhanced antibacterial activity against E. coli without too much toxicity [36]. The synergy between cold atmospheric plasma and hydrogen peroxide was revealed to possess powerful and unique bacterial disinfection properties in a recent study. When applied to notoriously difficult-to-kill bacteria like *Enterococcus faecalis*, the combination of cold plasma and H_2O_2 got the job done and left tissues (the target of the treatment) in a state that was conducive to healing. Dramatic improvements were noted in the efficiency of this plasma plus H_2O_2 approach compared with using either component alone [37].

6. CONCLUSION

Hydrogen peroxide plays a certain role in biofilm formation as an antimicrobial agent and impacts biofilm formation. Its ability to disrupt biofilm biomass is mediated by inducing oxidative stress. Role of H2O2 in eradicating the biofilm by preventing the initial adhesion of bacteria and maturation of biofilm. The role of H2O2 in biofilm production and bacterial resistance to antibiotics call for the redesign the treatment strategies. The different concentrations and duration of exposure of H2O2, the species of microorganism play a central role of H2O2 in biofilm formation. The synergistic effect of H2O2 and antibiotic increase the antibacterial effect of antibiotic and help in improving the antibacterial effect of antimicrobial agents. Emphasizing collaboration between microbiologists, healthcare practitioners, and researchers will pave the way for innovative and effective solutions to the ongoing problem of biofilm-associated infections.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical Approval

This review was approved by the Scientific Committee of the University of Baghdad, Baghdad, Iraq (1415; 16-04-2024).

Author contributions

Hwazen A. Shnyoor: Investigation; Methodology; Project administration; Resources; Supervision; Validation; Roles/Writing - original draft; and Writing - review & editing.

Ayaid K. Zgair: Conceptualization, Data curation, and Formal analysis, Roles/Writing - original draft; Visualization and Writing - review & editing.

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