Evolving Technologies and Strategies for Combating Antibacterial Resistance in the Advent of the Postantibiotic Era
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Abstract

The challenges posed by the impending ‘post-antibiotic era’ have put forward urgent challenges to be overcome by providing new diagnostic and therapeutic regimes for improved diagnosis and treatment of bacterial infections. Antibiotic resistance and incurable bacterial infections are especially important in a society faced with rapid demographic changes. With very few new antibiotics in the drug development pipeline, not being able to match the pace of antimicrobial resistance (AMR) evolution, developments within other fields such as materials sciences and medical technologies are required to realize innovative antibacterial approaches. In this progress report, we present recent advances in especially nanotechnology-based approaches and their concomitant use with complementary antibacterial treatments. Synergistically improved antibacterial activity can be reached by considering novel, promising approaches such as photodynamic and photothermal therapy (PTT, PDT) as well as cold atmospheric pressure (CAP) treatments as complementary strategies to fight against antibacterial resistance. We describe how these novel technologies can be further improved especially by integration of nanomaterials into the currently applied single modal strategies against bacterial infections.
1. Introduction

Antibiotic resistance together with bacterial biofilm formation are the main challenges in the treatment of infectious diseases, currently leading to severe clinical outcomes and high mortality.\(^{[1]}\) Antimicrobial resistance (AMR) i.e. the ability of microbes (bacteria, parasites, viruses, fungi) to resist antimicrobial drugs was declared among the top ten global health threats by the World Health Organization (WHO) in 2019.\(^{[2]}\) The inappropriate and excessive use of antibiotics, i.e. antimicrobials targeted against bacteria (thus also known as “antibacterials”), conjoined with the challenges associated with infectious diseases, decrease the success of conventional antibiotic therapies and lead to multifaceted problems. Antibiotic resistance already presents a serious social burden and is responsible for an estimated 25,000 deaths per year in the EU.\(^{[3]}\) It does not increase only the risk of morbidity, but also leads to extra healthcare costs and productivity losses of at least 1.5 billion EUR each year.\(^{[3]}\) Comparatively, biofilms are composed of aggregated cells of bacteria adhering to living or inert surfaces or to each other in a self-produced extracellular polymeric substance (EPS). Biofilms constitute major treatment obstacles since bacterial cells in a biofilm matrix can display up to a 1000-fold increase in antibiotic resistance compared to planktonic cells.\(^{[4]}\) Moreover, biofilms are known to be the root of 80 % of all infections in the human body.\(^{[5]}\) They are difficult to treat due to the poor penetration of antibiotics through the EPS and the heterogeneity in their microenvironments, where some bacterial cells survive even under difficult conditions due to inherent resistance to antibiotics. Thereby, biofilm-related infections affect millions of people resulting in up to 550,000 deaths every year.\(^{[1]}\) Consequently, the incidence of antibiotic resistance and recalcitrant nature of biofilms in bacterial infections cause unfavorable treatment outcomes.\(^{[6,7]}\) Consequently, infectious diseases are again on the rise, especially those that can no longer be treated using the previously discovered antibiotics. The terrifying scenarios of AMR is predicted to cause almost 10 million deaths per year by 2050 and a total GDP loss of $100.2 trillion by 2050 if appropriate actions are not taken.\(^{[8]}\) Without urgent action, we are heading towards what the WHO has coined the ‘post-antibiotic era’, in which infections that have been treatable for decades can kill again. Henceforth, it is imperative to come up with new solutions and technologies to overcome the current failures in infectious
disease treatments and optimize therapeutic, curative or preventive measures against bacterial infectious diseases.

Nanoparticles (NP) have emerged as players to be reckoned with in the treatment of infectious diseases, especially through the knowledge gained from the research in oncology-related nanomedicine. The unique properties of nanomaterials compared to their bulk forms render them favored also for antibacterial therapies. In accordance to the classically considered material properties that emerge when a material enters the nanoscale, including optical, magnetic, electrical and quantum effects; nanomaterials can be designed to represent inherent antibacterial properties that are not expressed in their bulk form. Furthermore, fast and sensitive bacterial detection can be enabled with nanoparticle-based approaches. In addition, nanoparticles offer discrete advantages as antimicrobial drug delivery systems. They can be designed as targeted, environmentally responsive, combinatorial antibacterial delivery systems. Nanomaterials as a platform can offer adjuvant solutions by integrating multiple antibacterial modes of action against the pathogenic bacterial species. In the quest of finding new approaches to serve for the shortcomings of conventional antibacterial therapies, NPs together with novel treatment regimens such as photodynamic therapy and plasma medicine, are foreseen as promising antibacterial strategies. The scope of this progress report is to emphasize the need of novel biomedical approaches within the field of antibacterial treatments, and to report on the advances in the field of antibacterial therapies with the emphasis on detection, prevention, and elimination of bacterial infections with different nanomaterial designs and concomitant plasma medicine and antimicrobial photodynamic therapy as novel approaches in combating antibacterial resistance and bacterial infections.

1.1. Conventional antibacterial strategies

The discovery of antibiotics have transformed modern medicine and saved millions of lives. The “golden era” of antibiotics between the 1930s to 1960s gave rise to many antibiotic drugs. However, inconsiderate consumption of antibiotics have inclined the emergence of antibiotic resistance. Despite the
co-occurrence of antibiotic resistance, antibiotics have played a pivotal role in achieving major advances in medicine and surgery. [14]

Antibiotics are designed to block crucial processes in microbial cells selectively, and they can be either natural products or man-made synthetic chemicals.[15] Antibiotic compounds have been modified to improve their actions against infectious diseases, leading to the development of semisynthetic agents.[16] Antibiotics are mostly designed to block protein or RNA synthesis, to inhibit DNA replication, or to attack the bacterial cell wall.[17] However, the microorganisms have the ability to develop resistance mechanisms to protect themselves against the antibiotics; this is the phenomenon known as antibiotic resistance (Figure 1).

There are several inherent ways of bacteria to inhibit antibiotics: modifying an antibiotic or the target of the antibiotic to block their interaction, blocking some metabolic pathways or changing the mechanism of efflux/influx pumps.[17] To overcome the failures of individual antibiotics for the treatment of bacterial infections, sequential or concomitant treatment regimes are employed. The use of concomitant antibiotic treatments refer to using different antibiotics at the same time, and are assigned to ensure treatment response when resistance is prevalent.[18] In essence, concomitant antibiotic treatments strategies are employed in order to enable bacterial attack on many fronts, making it significantly more difficult for microbes to develop resistance simultaneously toward all modes of action.[19,20]
Figure 1. Main antibiotic targets and associated mechanisms of resistance. Reproduced from ref.[14]

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Growth factor analogs, sulfa drugs and quinolones are commonly depicted as better options compared to conventional antibiotics.[21] They have a tendency to exhibit better selective toxicity against pathogens. When compared to the above-mentioned strategies with conventional antibiotics, they have a capability to influence deeper infections and induce less side effects on patients. They fight pathogens by blocking the mechanism of growth factor utilization in bacterial cells, leading to growth inhibition. Quinolones are not actually growth factor analogs, and thus, they do not compete with growth factors. They bind a specific bacterial enzyme, which has a specific role in DNA synthesis inside cells; thereby blocking the DNA synthesis mechanism.

Antiseptics are considered the other conventional option and an alternative for antibiotics, due their broad spectrum of microbiocidal effects for the management of localized superficial skin infections.[21,22]
Povidone iodine, polihexanide, chlorhexidine and octenidine are among the commonly used antiseptics to destroy pathogens or inhibit their growth. In general, antiseptics may have multiple targets during the inhibition of bacterial growth. However, these chemical agents can cause skin irritation, especially in their use for wound healing. In the worst case, this mode of action can even reverse the wound healing process; for instance, the absorption of iodine compounds by living tissue may induce acidosis or hyperthyroidism. In addition, these chemical agents cannot be considered as sufficient antibacterial tools because of their inability to penetrate deeper into tissues. Therefore, these may be preferred as good disinfectants to decontaminate non-living objects. Pathogens have not yet developed any known resistance mechanism against these antibacterial chemical agents per se, but their limitations lie in their bactericidal action and disadvantages associated with inducing harm to healthy living tissues.

Silver preparations are also recognized as a type of conventional antibacterial treatment. Silver is known to be biologically active when it is dissolved into its monoatomic ionic state (Ag⁺), i.e. when it is soluble in aqueous environments. The antibacterial action mode of silver preparations have been defined as follows: i) the formation of pores and punctures in the bacterial cell wall of bacteria due to the reaction of silver cations with the peptidoglycan components; ii) both inhibition of cellular respiration and disruption of metabolic pathways due to the entrance of silver ions into the bacterial cell and iii) disruption of bacterial DNA and replication cycle. The current trends in using silver ions is also focused on their usage as adjuvants for potentiating antibiotic toxicity, despite that there is little doubt this very ancient antibacterial metal can lead to something viable within the context of the multiple antibiotic resistance crisis.

Another commonly used strategy of eradicating infections for the topical co-occurrence of infections is surgical removal. Surgical removal is nevertheless ineffective for deeper lesions, as listed along with other disadvantages in Table 1; and there is a high probability to miss out some parts of infected tissue during surgery. In addition, surgery always causes risks of elevated pain and discomfort for the patient.

Table 1. Comparison of the conventional antibacterial strategies according to their mechanism of action and their adverse effects on the human body.

Consequently, while the pharmaceutical industry has struggled to keep up the development of new drugs that can meet the heightened requirements set by the voluminous spread of antibiotic resistance; the problems associated with conventional antimicrobial strategies have motivated researchers to concentrate on the development of novel, convenient and inexpensive treatments for fighting pathogens and infectious diseases from less conventional angles.

### 1.2. Novel antibacterial strategies

Medical technologies with the focus on antibacterial treatments are in a unique position to address several challenges when the declined investments in antibiotic drugs driven from older antibiotics, cost, uncertainty of the development process, and limited reimbursement incentives are considered.\[33\] Research efforts in medical sciences have been directed towards preventing AMR and providing broad-spectrum activities. Apart from the chemical strategies, physical antibacterial strategies with a multitude of antibacterial mechanisms and destruction of microbial growth are predominantly advantageous. They offer
novel and promising solutions while acting to synergistically improve the impact of antibiotics and natural antimicrobial compounds.

To date, a number of approaches with dominant physical antimicrobial effects has appeared on the medical sciences scene. Among these, cold plasma, photodynamic therapy, and nanotechnology offer potent and broad-spectrum antimicrobial effects. Encouraging the use of these novel antibacterial treatments may help to reduce inappropriate use of conventional antibiotics and thus, reduce the potency of antimicrobial resistance. Antibacterial nanotechnology, antibacterial photodynamic therapy (aPDT), and antibacterial plasma treatments are attractive technologies to be utilized as stand-alone treatments as well as to create combinatory therapies for multimodal attack on the bacteria. These novel technologies will thus be discussed below in order to highlight the recent advances in these particular fields.

1.2.1. Nanomedicine-based antibacterial treatments

The advances in nanotechnology-based approaches has brought forward an ample number of novel tools enabling unique solutions in antibacterial treatments. To date, nanotechnology-based solutions have been employed to prevent, detect and treat bacterial infections\cite{34} as illustrated in Figure 2.
Figure 2. Examples of nanotechnology-based solutions that could help to prevent, detect or treat bacterial infections. A) The prevention of bacterial spread by trigger-dependent release of silver ions from surfaces of implants. B) A point-of-care compatible setting for rapid detection of bacterial species C) Sustainable delivery of antibiotics i.e. antibiotics encapsulated in enzyme-cleavable polymers. D) Removal of pathogens from blood i.e. magnetic particles capture the pathogens and are separated from the blood by an external magnetic field. Reproduced with the permission[^34] Copyright © 2015, Springer Nature.

Antimicrobial nanomaterials have great potential to fight against infectious diseases. The high surface area-to-volume ratio of nanoparticles potentiates new physical (such as mechanical, optical and electrical) and chemical properties, and enables intimate interaction between the microbial membranes and the surface of the nanoparticles,[^35] which can furthermore be flexibly fine-tuned by surface functionalization methods. Nanoparticles (NP) can exert antibacterial activity through variable mechanisms, such as direct interaction with the cell wall, inhibition of biofilm production, triggering of both innate and adaptive immune responses, production of reactive oxygen species (ROS), and induction of intracellular events (interaction with DNA hinder protein function) as presented Figure 3.[^36]

![Figure 3](image_url) Schematic depicting of the antibacterial action modes of nanoparticles. Reproduced by permission[^36] of The Royal Society of Chemistry.
Metal / metal oxide nanoparticles have become widespread in medical antibacterial usage in recent years. They may inherently exhibit particularly toxic effects,\textsuperscript{[37,38]} that bacteria alas can develop resistance against.\textsuperscript{[39–41]} Meanwhile, progress in supramolecular chemistry for the design of biocompatible organic NPs also for antibacterial treatment has displayed some successful results, especially with regard to cationic polymeric NPs.\textsuperscript{[42,43]} Nevertheless, the challenges associated with the chemical and colloidal stability of organic NP have been pointed in the literature findings.\textsuperscript{[42,44]} When the advantages and disadvantages of inorganic and organic NPs are considered, the combination and hybridization of organic and inorganic constituents to form nanoparticles represents a new strategic approach for fighting bacterial infections\textsuperscript{[45]} by reducing the toxicity, antibiotic resistance and cost aspects of inventing new antibiotic molecules.\textsuperscript{[46,47]}

Tailoring the physicochemical properties of nanomaterials render them promising candidates to provide improved antibacterial efficacy against multidrug-resistant (MDR) bacterial infections.\textsuperscript{[47]} With the aid of nanomaterial engineering, crucial parameters such as the size, composition, and surface chemistry of the NPs can be tuned and play important roles in interacting with multiple bactericidal pathways.\textsuperscript{[48]} Especially the size and surface chemistry of NPs are critical properties to provide multimodal acting mechanisms against MDR. Considering already defined possible action modes of NPs, Huang et al. clearly showed how the multiple bactericidal acting modes of Se-NPs could be tuned by only changing the size of the NPs between ~40~200 nm. The Se-NPs were shown to induce size-dependent multimodal mechanisms of action, including depleting internal ATP and inducing ROS production, and disrupting membrane potentials. Specifically, among the 5 differently sized (43, 81, 124, 161, 205 nm) NPs, the smallest 43 nm-sized Se-NP caused bacterial membrane disruption and altering of the potential of the bacterial cell membrane, together with increased ROS generation and depletion of intracellular ATP. Instead, the bigger sized 83 nm Se-NP led to interfering with the metabolism of the bacteria through depletion of intracellular ATP, and more increased production of ROS. Comparatively, the 124 nm Se-NPs did not exhibit significant effects neither on promotion of ROS production nor change of membrane potential. No significant
antibacterial effect could be observed with the larger sized Se-NP. Overall, multiple bactericidal pathways can be achieved by only altering the size of NP; however, the most bactericidal effect was achieved with the 81 nm sized NP, which still had slightly less impact on providing multiple bactericidal pathways compared to smaller sized 43 nm Se-NP.

The impact of surface chemistry in providing bactericidal effect depends on the type of NP interaction with bacterial cells. Usually multiple interaction mechanisms are simultaneously at work, such as electrostatic attraction, hydrophobic and receptor-ligand interactions, and van der Waals forces between the NP and bacterial membrane.\[^{49}\] When the interaction leads to NP internalization, loss of membrane integrity takes place most likely due to intracellular oxidative stress.\[^{50}\] This event also triggers the sequential cell apoptosis\[^{51}\] by inducing gene expression levels of oxidative proteins, which is a key mechanism in bacterial cell apoptosis. Further, ROS can mediate DNA damage,\[^{52}\] attack proteins and depress the activity of certain periplasmic enzymes that are essential to maintaining normal morphology and physiological processes in bacterial cells.\[^{53}\] There have been attempts to investigate the impact of NP surface chemical groups to predict the interactions with bacterial cell membranes and thereby resulting bactericidal pathways. Studies have revealed that cationic functional groups on NP lead to strong interactions between NPs and the bacterial surface, while anionic functional groups with carboxylate headgroups compete for H-bonding interactions with cell wall components, disrupting their structural integrity and causing cell lysis. The surface modification of NP can thus be exploited as a tool box to trigger sequential bactericidal pathways, which usually start with the bacterial membrane disruption that can create a domino-effect leading to bacterial inactivation in the treatment process.\[^{54}\] To the best of our knowledge, few studies have evaluated the combinatory effect of morphology and surface modification of NP. For instance, the impact of type and degree of surface modification together with the shape of NP on providing multiple bactericidal effects have been evaluated. As presented by Sen Karaman et al.\[^{55}\] by tuning the NP aspect ratio, doping with Ag\(^+\) and coating with a antibacterial polymer surface layer, their results revealed that the NPs with the highest aspect ratio and
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organic surface modification portion also had the greatest impact to attack the targeted bacteria on many fronts, which can be foreseen as a crucial strategy to inhibit the development of resistance.\textsuperscript{[56]}

In addition, the use of NP as vectors for conventional antibiotics adds advantages related to their small and controllable size, their ability to deliver antibiotic drugs and several other therapeutics integrated into one nano-system.\textsuperscript{[57]} When NPs are used as delivery vehicles, advantageous features including enhancement of (loaded) drug solubility and stability,\textsuperscript{[58]} simple synthesis procedures and tunable biocompatibility can be provided.\textsuperscript{[47]} Increased antibiotic resistance among bacteria constitutes a driver for new classes of synthetic and natural nanomaterials to battle resistance mechanisms in varying ways. Coupling of nanoparticles and naturally derived antimicrobials (or synthetic antibacterial compounds) to inhibit the activity of bacterial efflux pumps, formation of biofilms, interference of quorum sensing, and possibly plasmid curing, are just some of the strategies to combat multidrug resistant bacteria.\textsuperscript{[47]}

Preparation of antimicrobial NP is considered cost-effective compared to the synthesis of molecular antibiotics. This has given rise to a new term ‘nanoantibiotics’, indicating that the nanomaterials in question possess antimicrobial activity by themselves or provide elevated efficacy and safety of antibiotic administration. Overall, the utilization of nanoantibiotics has shown great potential in the battle against infectious diseases due to multiple advantages:\textsuperscript{[58]} 1) improved solubility of antibacterial compounds, 2) uniform distribution in the target tissue, 3) sustained and controlled release, 4) enhanced cellular internalization, and 5) minimized side effects. Recent progress in the field of nanoantibiotics, their utilization in diagnosis and prevention of bacterial infections, as well as their combinatory utilization with other novel technologies (e.g. photodynamic and cold atmospheric plasma treatments) against bacterial infections will be considered in the following sections.

1.2.2. Antimicrobial phototherapy

Phototherapy is a minimally invasive method and considered a promising strategy in the treatment of bacterial infections. The provided high bactericidal effects by the action mechanism of phototherapy have received considerable attention. As a therapeutic approach, photothermal therapy (PTT) has been used to
treat bacterial infections making use of light-absorbing materials with high light-thermal conversion efficiency under the irradiation of an external light source (usually near-infrared light). PTT depends on local heating of biological tissue by the absorption of light energy, which will lead to irreversible tissue damage via denaturation of protein and collagens, permeabilization of cell membrane, and so forth. However, because of heat dissipation, PTT may influence neighboring cells and result in death of these cells, too.\textsuperscript{[59,60]} Several materials (such as precious metal NPs, transition metal sulfide, carbon nanomaterials, and upconversion NPs) are utilized as agents for PTT to promote the increase in local temperature of the targeted site, resulting in necrosis.\textsuperscript{[61]} The primary problem that remains to be solved has been reported as the antibacterial efficacy can reach 90\% only in temperature conditions of around 85°C.\textsuperscript{[62]} This quite excessive temperature can disrupt the nature of tissues and lead to other diseases or problems. Thus, this has been considered the greatest challenge of PTT, which requires attempts to minimize adverse effects on healthy cells.\textsuperscript{[59]} In the current attempts of overcoming the challenges of antibacterial PTT treatments, heating can either be used to deliver thermal energy to a localized area or, alternatively, throughout the drug carrier, thereby causing the destruction of the encapsulating coating and triggering the release of the loaded therapeutics.\textsuperscript{[63]}

Photodynamic therapy (PDT) has been used to diagnose and to treat disease through photosensitizers to produce reactive oxygen species (ROS) - in particular singlet oxygen under appropriate irradiation. PDT is also a therapeutic approach to fight bacterial infections. The three main components of PDT can be defined as a light-sensitive drug (photosensitizer), light, and oxygen molecules. The acting mechanisms involve the steps presented in Figure 4: (1) irradiation of the photosensitizer (PS) by a light source, (2) activation of the PS due to absorbed light energy, and (3) transferring of absorbed energy to molecular oxygen to produce ROS as antimicrobial agent.\textsuperscript{[64]} These compounds cause oxidative stress on cells and induce apoptosis or necrosis in the organism.\textsuperscript{[65]} The treatment of microbial cells with PDT has been carried out for 100 years, since researchers noticed that cells of Paramecium spp. stained with acridine orange were destroyed upon exposure to bright light.\textsuperscript{[66]} Since then, PDT has primarily been developed as a treatment for cancer, ophthalmological disorders, and for use in dermatology.\textsuperscript{[65]} Temperature increase,
which may cause irreversible tissue damages and adverse effects in neighboring healthy cells is thus not likely to occur in PDT.\textsuperscript{[60]}

Figure 4. Schematic representation of the mechanism of antibacterial photodynamic therapy (aPDT).

In recent years, researchers have revived the antimicrobial effects of PDT, detonated antimicrobial photodynamic therapy (aPDT) due the emergence of antibiotic resistance among pathogenic bacteria. Furthermore, recent findings have revealed that aPDT can be suggested as therapy for a variety of localized microbial infections.\textsuperscript{[67,68]} aPDT on localized superficial infections is convenient and effective, and it has fewer side effects when compared with conventional antibacterial strategies and PTT.\textsuperscript{[67]} The effectiveness of PDT depends on the PS features, the localization of the PS in/around the bacterial cell during light application, irradiation at the appropriate wavelength for PS and the structure of the bacterial cell wall.\textsuperscript{[69]} The most commonly used PSs are porphyrins, chlorines and phthalocyanines.\textsuperscript{[70,40]} According to a report by Abrahamse and Hamblin, several PS such as HpD (haematoporphyrin derivative), Photofrin, PPIX (Protoporphyrin IX), Verteporfin (benzoporphyrin derivative), Radachlorin (now Bremachlorin), Fullerenes, Temoporfin, or Foscan (mtetrahydroxyphenylchlorin) have recently received clinical approval.\textsuperscript{[71]} Commonly used PSs have a broad
absorption band between 400-430 nm and a narrower absorption band above the wavelength of 550 nm. Still, wavelengths around and above 600 nm are more advantageous in PDT applications because of its higher quantum yield and deeper penetration ability into biological tissues.\[^71\] For an effective aPDT, the appropriate wavelength should be selected according to the content and depth of the tissue, and the PS should be have a high absorption capacity for this specific wavelength to result in high singlet oxygen quantum yield to create the therapeutic effect.\[^71,72\] In addition to this, the PS should be chemically stable during storage and cost-effective. PSs should have an absorption band in the red-near infrared spectral region (650-800 nm), accumulate specifically at the lesion site, show reasonable excretion and not exhibit toxicity in the absence of illumination.\[^72,73\] However, considering the existing PS, none of them encompasses all the required features of an ideal PS. For instance, chlorines generally have high stability, while their penetration ability is poor and their ability to act on gram-negative bacteria is limited.\[^74\] Indocyanine green is known to be effective in deeper tissues because of the deeper penetration capacity of the wavelength required for its illumination; however, their cellular interactions are limited due to their anionic structure.\[^75\] Although porphyrins have advantageous cationic structure and modular chemical structure, their aPDT activity is weak due to the low absorption band.\[^76\] By combining photosensitizers, a relevant light can stimulate material to produce ROS and kill bacteria. However, the hypoxic environment is regarded the limitation of the PDT efficiency \textit{in vivo} due to the lower ROS yields, which is a great challenge for PDT. Therefore, the main limitations of PDT are associated with PS distribution and limited penetration of the laser to deep tissue.\[^77\] PS delivery is one of the major challenges in aPDT due to their hydrophobicity, partial stability, short half-life, poor selectivity between the unhealthy and healthy cells and toxicity in the absence of illumination, as well as poor penetration through the tissues.\[^78,79\] Here, nanoparticles can offer significant advantages to improve the PDT beyond its inherent limitations, by providing PS delivery to the site of interest and manipulating the physical and chemical properties that are required to achieve biological interactions.\[^80\] To overcome the challenges of PDT, two different strategies can be followed in order to provide singlet oxygen production that is related to the efficacy of PDT. The first strategy is forming PS encapsulated in biodegradable/non-
biodegradable NP carriers, and the second one involves combining PS with inorganic NP with different optical properties i.e. NPs that can match the working region of PS. Therefore, the concomitant use of nanomaterials and PDT has made a significant contribution to the efficiency of PDT in antibacterial treatments. The combinatory therapy of aPDT and nanomaterials could be depicted as a promising regime against resistant bacteria, because of the multi-target killing mechanism; as will be further discussed in section 2.3.1 along with recent literature findings.

1.2.3. Antibacterial cold atmospheric plasma treatments

As stated earlier, research efforts in biomedical sciences have been directed to develop physical methods with broad-spectrum effects to combat microbial pathogens. Up to date, no method with dominant physical antimicrobial effect have appeared in the scene of biomedical sciences, except UV photons that were not conveyed to clinical practice due to safety reasons. However, as a physical antimicrobial technology, cold plasma offers potent and broad-spectrum antimicrobial effect due to the generation of strong antimicrobial components such as ROS, RNS, and free radicals during the formation of plasma discharge. These have effects on the multiple targets in microbial cells and induce lipid peroxidation on the cell membrane, oxidizes cell walls and DNA damage. Furthermore, cold plasma provides selectivity between prokaryotic and eukaryotic cells, which makes it a safe method to apply for the eradication of infections on living tissues. In addition, cold plasma technology is also capable to modify various materials chemically to make them acquire broad-spectrum antimicrobial effects, which makes it applicable for the prevention of both infections and biological decontamination.

Antimicrobial treatments by cold atmospheric plasma (CAP) is one of the most prominent applications of CAP, and the first reports date back to the last decade of the 20th century. Since then, the antimicrobial effect of CAP on bacteria, bacterial spores, fungus, protozoans, virus, and even prions have been investigated. The state change of matter is mainly governed by the energy input to the matter; and the continuous energy input to the gas leads to ionization of the gas and formation of plasma, known as the fourth state of the matter. When the electrical energy is applied to a gas, firstly, free electrons are
accelerated under the influence of the electric field causing them to collide with heavier gas atoms/molecules which, in turn, causes the removal of electrons from gas atoms and ionization of the gas, i.e. formation of plasma.\[^{[86]}\] Depending on the thermal equilibrium in between electrons and heavier particles, plasma is classified as hot (or thermal) and cold (or-non-thermal) plasma. By virtue of applicability of cold plasma at atmospheric pressure on heat-sensitive materials such as living tissues, organisms and biomaterials, non-thermal plasma became an emerging technology for various biomedical applications and created a new field of “Plasma Medicine”.\[^{[87]}\] Furthermore, as a clinical concern, CAP has been reported to have broad-spectrum antimicrobial activity on even biofilm forms of multidrug-resistant bacteria, which makes it a promising tool to combat superbugs.\[^{[88]}\] CAP could be applied to the substrate of interest in various ways. In direct CAP treatment, the substrate (e.g. skin) that is being treated acts as a counter electrode and the CAP is generated in between the skin tissue and plasma electrode, usually in ambient air as the treated tissue is present in the ambient atmosphere. In indirect CAP treatment, the plasma discharge is generated remotely from the target tissue and plasma products are carried towards the tissue via a gas flow. Indirect CAP treatment is also called plasma jet, and this modality involves the use of jet electrodes and surface discharge electrodes that can generate plasma of different gases including air, helium argon etc. and/or their mixtures.\[^{[89]}\] CAP treatment is capable of modifying various materials including different liquids, gels, polymers, and metals, to make them potential novel tools against clinically relevant pathogens in two different ways.\[^{[90]}\] CAP treated materials can acquire antimicrobial effect that is comparable to that of CAP by itself. In addition to this, the surface of CAP treated materials may be modified in a way that prevents and/or reduces microbial adhesion and subsequent biofilm formation.\[^{[91–94]}\]

The antimicrobial effect of CAP is primarily linked to ROS and reactive nitrogen species, RNS (or collectively reactive oxygen and nitrogen species or RONS), and to a lesser extent to free electrons, ultraviolet (UV) photons and electromagnetic field that are generated during formation of plasma.\[^{[49,95,96]}\] The antimicrobial effect of CAP rises mainly from the induction of oxidative and nitrosative stress that has multiple cellular targets, e.g. cell membrane, DNA and proteins. CAP induced oxidative and nitrosative stress
show their first effect on the cell as membrane damage, which is primarily due to lipid peroxidation that might be accompanied by lipid nitration according to some reports.[97] Also, damage of membrane proteins when CAP or CAP treated substrates were exposed to microbial cells, has been shown.[90] When CAP generated ROS and RNS penetrate microbial cells, single and double-strand breaks and loss of protein and enzyme function due to either oxidation or nitration of amino acids were reported.[98] Remarkable antimicrobial efficacy of CAP conveyed this technology to the clinical scene mainly for the treatment of infected wounds with promising outcomes. Currently, several CAP devices that are certified as medical devices are in clinical use for severe wound treatments.[99]

The species formed with CAP can be applied to synthesize biologically important nanomaterials or can be used with nanomaterials for antibacterial treatments.[100] Recently, researches have been using plasma technology as a prominent “green” synthesis method for nanomaterials and investigating their antibacterial properties.[101] A combination of nanomaterials and cold plasma is also gaining attention in order to provide synergistic effects and better treatment efficiency in biomedical applications.[100,102] In medical applications of CAP and NP for antibacterial treatments, synergetic effects in terms of reactivity, selectivity against pathogens, their toxicity to healthy cells and tissues, and their penetration to the lesion site regions are aimed for.

When conventional and novel strategies are considered, medical technology solutions are emerging in response to the antimicrobial resistance challenge. Literature reports show that concomitant medication with different antibiotics provides better efficacy in the eradication of bacterial infections compared to sequential or hybrid therapies. [103,104] However, the current strategies have reframed from using antibiotics, and therefore, the researchers have a tendency to combine medical technologies for diagnosis, prevention, and therapy in combating bacterial infections.

2. Nanomaterial-integrated diagnosis, prevention, and therapy of bacterial infections

2.1. Nanomaterials for bacterial detection and identification
As mentioned, the inappropriate and excessive use of antibacterial agents and antibiotics promotes the appearance of new resistant strains. This fact has driven the interest of the scientific community to come up with rapid detection and identification of microorganisms in the field of clinical diagnostics and monitoring of pathogens. The current world standards for pathogen diagnosis, including culture, enzyme immunoassay and polymerase chain reaction (PCR), are considered as cost-prohibitive and often takes 2 to 4 days to perform.\textsuperscript{[105]} On one hand, biosensors are known to provide numerous advantages such as reduced hands-on time and high-throughput screening possibilities over existing techniques in pathogen diagnosis.\textsuperscript{[106]} On the other hand, biosensor systems cannot detect bacteria at low concentrations without bacterial pre-enrichment of the sample.\textsuperscript{[107]} This becomes quite critical, since a single cell may lead to several health risks. Hence, scientists have devoted themselves to creating ultrasensitive detection approaches in infectious agents detection for bacterial sensing in complex biological matrices.

Nanotechnology can eliminate the difficulties in bacterial biosensors on intrinsic heterogeneity, development of genetic circuits, durability of the reagents, contamination during storage periods and required skilled labor by providing (i) enhanced measurements for detection; (ii) immobilized and automated cultivation for detection environments; and (iii) portability and facile handling.\textsuperscript{[108,109]}

In the design of effective sensors, commonly two key issues are considered. The first one is the limit of detection (LOD), which should be between $10^4$-$10^2$ cells/mL to meet the need of clinical applications.\textsuperscript{[110]} The second one is the readout, which should not require expensive instrumentation. Many research groups pursue this goal, since existing biosensors do not fulfill this requirement. The requirement of reliable and sensitive targeting of pathogens has endorsed investigations on the potential of nanoparticles and their integration into biosensor systems. Nanoparticulate systems offer a versatile platform that can offer inherent features depending on the nature of the nanoparticles to be integrated. In addition, they can be functionalized, which broadens their potential and opens up new possibilities for customized bacterial detection and identification. It is envisaged that integrating optical, electrochemical and magnetic
nanoparticles in bacterial biosensors will provide enhancements as point-of-care systems, due to their adaptability in different configurations.\textsuperscript{[107]}

Optical properties of nanoparticles such as reflection, transmission, absorption, and light emission, are most likely different from those of bulk materials. These properties are frequently exploited in biosensing approaches.\textsuperscript{[111]} In the current approaches with biosensors, researches have exploited both the inherent and added optical properties of NPs to provide ultrasensitive bacterial detection. For instance, gold nanoparticles (AuNP) have been used over a wide range of biological sensing approaches, due to their surface plasmon band localization in the visible spectrum, ease of synthesis, and various functionalization capabilities.\textsuperscript{[112]} One of the recent progress on AuNP integrated bacterial detection includes naked-eye detection of \textit{E. coli} from urine samples of patients with urinary tract infection (UTI).\textsuperscript{[113]} With the sensing ability of the designed AuNP, a linear relation in the range of $1 \times 10^3$–$4 \times 10^3$ cells/mL with a detection limit of 100 cells/mL could be provided. In this particular design, the sensing mechanism of AuNP directly depends on surface modification of AuNP with highly positive (-NH$_2$) cysteine binding to and high negative charge caused by the endotoxin or lipopolysaccharides found in the outer cell wall of \textit{E. coli} 0157:H7. The electrostatic interaction leads to visible color change of the AuNP + bacteria solution from red to blue, providing a simple detection by the naked eye. This straightforward detection method could be used as a self-screening method by patients suspecting UTI for warranting further medical attention after integration of the nanoparticles into urine test strips. Recently, the concept of integrating the sensitive detection to point-of-care devices have been realized by Cho et al.\textsuperscript{[114]} Researchers have designed smartphone-based, sensitive detection of UTI and gonorrhea by microfluidic paper analytical device (µPAD) for early detection of bacterial species (\textit{E. coli} and \textit{N. gonorrhoeae}) caused UTIs, and some sexually-transmitted diseases (STDs) by using optical properties of nanoparticles\textsuperscript{[114]} The designed µPAD encompass pre-loaded and dried \textit{anti-\textit{E. coli}} or \textit{anti-\textit{N. gonorrhoeae}} antibodies conjugated to polystyrene latex NPs in the center of a microfluidic channel. The readout was obtained by ambient light source and image acquisition with Mie scatter simulations and software by Laven. The experiments were conducted on a specific bacterial concentration of...
10^5 CFU/ml; however, the authors have stated that significant signals could be observed starting from 10 CFU/mL. The sensitive detection obtained with the aid of optical properties of NPs is advantageous from the point of providing low cost and point-of-care devices for early detection of infectious diseases. This, in turn, enables appropriately directed therapy to be initiated, hence improving the patient healing and aids in retarding the development of persistent or AMR infections.

In recent investigations by Oscar et al. enzyme-NP assemblies have been prepared to provide rapid and sensitive colorimetric sensing of bacteria.\textsuperscript{[110]} With this system in a solution platform, bacteria concentrations as low as 100 cells/mL could be determined in seconds. Transfer of this investigated methodology on a test strip has provided a visual sensitivity of 10^4 bacteria/mL, which has become a potential tool for field applications. More recently, in 2019 Wang et al. have integrated both fluorescent and magnetic NPs in order to provide an ultimate sensing microfluidic biosensor for online and sensitive detection of \textit{Salmonella typhimurium}, as shown in Figure 5 with the same motivation as above.\textsuperscript{[115]} This recent progress mainly focuses on serving the need of clinical requirements of effective biosensors. The microfluidic biosensor system encompasses magnetic nanoparticles (MNPs) modified with monoclonal antibodies against \textit{Salmonella typhimurium}. These were first used to separate the target bacteria from the sample background, which then reacted with the fluorescent particles (FMSs) modified with polyclonal antibodies (PAbs) against \textit{Salmonella typhimurium} to form the MNP-bacteria-FMS complexes (fluorescent bacteria). The biosensor has the ultimate sensitivity of detecting \textit{Salmonella typhimurium} ranging from 1.4 × 10^2 to 1.4 × 10^6 CFU/mL, which has a lower detection limit of 58 CFU/mL and is based on immunomagnetic separation, fluorescence labeling and smartphone video processing. The separation and detection strategies integrated biosensing device for is critically important in order to provide bacterial species enrichment and their strict identification. Thereby early, rapid detection of the causative pathogens can be identified and further antibiotic therapies can be planned without delay.
In addition to exploiting the inherent optical properties of NPs for bacterial detection and identification, researchers have doped polymeric or ceramic nanoparticles with organic fluorophore dyes. By the encapsulation of fluorophore dyes, a protective shell around the organic dyes can be provided as dye isolator, which not only suppresses photobleaching but also yields almost $10^4$ times more fluorescence signal compared to the organic fluorophore in free molecular form. When biorecognition takes place with organic dye molecule integration into NPs, greatly enhanced fluorescence signal could be obtained. By this way, ultrasensitive analyte determination can be provided. The early use of this strategy have been carried out in order to manage rapid single bacterial cell quantitation by using fluorescent dye incorporated and antibody conjugated silica nanoparticles, as demonstrated by Zhao et al. In their study, they have achieved detection of $1–400$ E. coli O157:H7 cells per sample in 20 minutes through antibody-antigen interaction and recognition in spiked ground beef samples. The prepared NPs could emit an extremely strong fluorescent signal and thus enable enormous signal amplification for ultrasensitive bacterium detection, which is otherwise undetectable with existing labeling technologies. The accurate and strict identification of...
infectious bacterial species is vital in order to initiate appropriate antibiotic therapy. The designed ultrasensitive fluorescent nanoprobes by Zhao et al can be ascribed to inform rapid, efficient diagnostic tests in order to provide accurate and prompt diagnoses and make evidence-based prescribing and dispensing in clinical/pharmacy practices, as also put forward as critical by the WHO antimicrobial global action plan.\textsuperscript{[118]}

Besides the identification of bacterial species, researchers have explored new fluorescent NP probes for the determination of bacterial survival as live/dead, which is beneficial for accurate determination of bacterial susceptibility. In a study by Lin et al. eco-friendly bacterial extracellular polymeric substrate coated carbon dots (CDs-EPS605) were prepared and tested for the discrimination of the live/dead status of \textit{M. luteus} (Gram positive), \textit{S. subtilis} (Gram positive), \textit{E. coli} (Gram negative), and fungus \textit{P. pastoris}.\textsuperscript{[119]} For all tested microorganisms, only dead microorganisms show strong florescence in the blue, green and red channels excited at 405, 488, and 552 nm, respectively, whereas no fluorescence was observed for the corresponding live ones. They have concluded their achievement as the live/dead cell distinction by bacterial extracellular polymeric substrate coated carbon dots being microorganism-universal, which owns distinguished merits including multicolor fluorescence emission property, negligible cytotoxicity, excellent photostability, low cost, and convenient staining process. These extracellular polymeric substrate coated nanoprobes can be a rapid and accurate determination of bacterial susceptibility especially during the invention of antibacterial compounds or materials added to evidence-based prescribing in clinical practices.

Bacterial separation and enrichment of causative pathogens from patients' biological samples is pivotal before onset of antibiotic therapy.\textsuperscript{[120]} However, usually antibiotic treatment is initiated based on clinical and epidemiological information and culture based diagnosis (blood culture) remains the reference standard to identify the causative pathogens in bloodstream infection, which can take a week of duration and still, up to 50% of suspected bloodstream infections occur with negative blood culture, which can delay adequate antibiotic therapy.\textsuperscript{[121]} For the sake of rapid onset of the therapy, new strategies are needed to be developed. In this regard, magnetic nanoparticles have found vast applications within bacterial separation and enrichment as well as bacterial detection \textit{in vitro} but also \textit{in vivo}, as presented in the scheme below.
In the concept of bacterial separation and enrichment, surface functionalization of magnetic nanoparticles with targeting moieties such as antibodies, narrow-spectrum antibiotics, antimicrobial peptides, and aptamers have been frequently employed, which can lead to prompt enrichment of the bacterial species from the biological samples. Consequently, quick onset of therapy can be provided without leading to a persistent bacterial infection, since it is well known that infection with an antibiotic-resistant pathogen is strongly associated with a longer delay in initiation of appropriate antibiotic therapy.

![Figure 6](image)

**Figure 6.** Magnetic nanoparticles for bacterial detection and therapy. Reproduced with permission from [122]

In order to skip the step of bacterial species enrichment from biological samples, ultra-low limit of detection (1 CFU/mL) have been achieved. An immunoassay of *Staphylococcus aureus* (S. aureus) with the aid of magnetic nanoparticles and liposomes was developed. The infectious doses of many pathogenic bacteria are as low as 10 cells; therefore, their investigations have great importance especially for detecting any possible infection transmission. The developed biosensing system encompasses cysteine (Cys)@liposome anti-*S.aureus* antibody decorated nanocapsules, and immunomagnetic NPs recognizing *S.aureus* cells, which are used in magnetic separation and, subsequently, liposome destruction was achieved.
The Cys molecules released from liposome nanocapsules were used both for colorimetric and fluorescent analysis with 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl), which is a non-fluorescent reagent reacted with aliphatic amines of Cys to form fluorescent and colored adducts. The provided amplification effect by the Cys from liposome nanocapsules destruction and the high capture efficiency of immunomagnetic NPs low detection limits could be as low as 10 and 1 CFU/mL for colorimetric and fluorescent detection, respectively. Another low limit of detection (10 CFU/mL) could also be managed with nanoparticle-aided electrochemical detection, which has recently been developed for the detection of *E. coli* and *Streptococcus pneumoniae*. In this approach, *E. coli* cells were decorated with silver NPs and the amperometric response for the AgNPs was measured and used for estimating the *E. coli* concentration.\footnote{127,128} In the other method, gold NP decorated *S. pneumonia* was subjected to conductivity measurements and allowed detection of low bacterial concentrations.\footnote{129}

Apart from the low limit of detection of the bacteria, the *in vivo* tracking of bacterial infection is also critical in order to provide an appropriate diagnosis. Within this concept, paramagnetic nanoparticles have been employed as MRI contrast agent for the assessment of antibiotic therapy. In the presented concept, the NPs underwent macrophage phagocytosis and could be followed in order to predict the treatment success.\footnote{130} The imaging modality integration for the tracking of bacterial infection and success of the employed therapy is important, and magnetic NPs are promising probes to provide it. The field is still limited to designs of theranostic magnetic NPs to provide for “follow, find, fight” strategies for bacterial infections.

Thus, development of nanoparticle-integrated bacterial detection and identification systems has been achieved over the past decade; which are critically important to prevent persistent bacterial infection occurrence, AMR infections, and providing rapid onset of therapy. However, there is still a great challenge in translation of the systems in practice. In order to realize clinical translation successfully, there is a need of making these devices suitable as point-of-care devices with suitable shelf lives, by evaluating the stability of the designed nanomaterials and properly providing them as complete kit packages. It is well known that NPs with complex designs (e.g. species selective tags modified NPs) have a tendency to aggregate in biological
media; which, in turn, can easily result in false positive or negative signals. To overcome these problems, researchers should focus on providing recognition agents on the surface functional groups of NPs that can also take a role in physical as well as signaling stability.

2.2. Nanomaterial-based preventive strategies against bacterial infections

Prevention of infection is a practical and evidence-based approach that can avert patients and people around the patients from being effected by avoidable infections, which can further provide elimination of morbidity and even mortality, saving costs, and diminishing the risk of antimicrobial resistance spread. Infection prevention has great importance for healthcare organizations because nosocomial infections are a huge burden, since infected patients require a prolonged hospital stay with possible life-threatening complications with additional costs for the healthcare organizations.\[^{131}\] The sources of nosocomial infections can be not only transmission among patients and health employees, but it can also be due to suppressed immune responses, surgical site infections, implants, and prostheses. These can lead to central line-associated bloodstream infections (CLABSIs), including catheter-associated urinary tract infections (UTI), and ventilator-associated pneumonia (VAP).\[^{132}\] In order to overcome the risks, the conventional strategies that are used are personal protective equipment, drugs and disinfectants, and antiseptics.

The growing interest in the design of antibacterial nanomaterials has also started to take part in infection prevention approaches.\[^{133}\] Numerous nanomaterials can be used alone or deposited onto surfaces; integrated into composites in order to provide disinfection solutions, inherently antibacterial hospital surfaces, surgical equipment, or patient implants.\[^{134}\] A very recent promising formulation of disinfection solution was provided in a recent study of Koklic et al.\[^{135}\] Koklic et al. produced copper-doped TiO\(_2\) nanotubes and used these for disinfection of plastic surfaces at room temperature, which resulted in a stable deposition resistant to multiple washing. Effective surface disinfection could be achieved due to the high photocatalytic activity of the TiO\(_2\) nanotube-coated surfaces when continuously illuminated with low-intensity ultraviolet-A (UVA) light. The prepared disinfection suspension of TiO\(_2\) nanotubes was successful to inactivate resistant
strains such as methicillin-resistant *Staphylococcus aureus* (MRSA) and Extended-spectrum beta-lactamase *Escherichia coli* (E. coli ESBL). Inhibition of up to $10^3$ microorganisms per cm$^2$ for 24 hours was achieved.

Inorganic or organic designs of nanostructured surfaces and coatings could inhibit bacterial adhesion on the surface of the site of interest.$^{[136]}$ Attractive results could be obtained with surface coating of medical devices, patient implants, and surgical equipment.$^{[137]}$ Nanoparticles deposited on surfaces can provide different mechanisms of action, as illustrated with the possible architectures of nanoparticles to serve for these therapies in Figure 7. Nanoparticle deposition can be carried out via physical or chemical deposition routes, the choice of which will have an impact on the extent of antibacterial effect duration.$^{[138]}$

![Figure 7](image)

**Figure 7.** (a) The features of multifunctional nanostructures and (b) the antibacterial nanostructured coating strategies on a substrate. (c) The possible preventive scenario by the nanostructured coatings. Reproduced with permission.$^{[136]}$ Copyright © 2016 Mohankandhasamy Ramasay and Jintae Lee.

The antibacterial action of deposited NPs on surfaces could be based on both repelling the adhesion of and killing the bacteria, as presented in Figure 9. Once the antibacterial NP coatings are deposited on implant surfaces, the surfaces should maintain their antibacterial properties within the therapeutic window.
until integration with the surrounding tissues, which can take up to several months; to prevent bacterial colonization from the hematogenous route. Recently, different methodologies in order to provide even and long-lasting antibacterial nanoparticle coatings have been investigated. In this regard, plasma treatment offers a rapid method for the covalent attachment of antibacterial content to the substrate without obstructing the bulk properties. Thin polymer films or coatings on surfaces with different plasma processes improve controlled loading and release of drug molecules; and CAP can lead to antibacterial surface activation and functionalization associated antibacterial activities. Therefore, there is a growing interest in the preparation of antibacterial nanocomposite films by CAP. In the literature, the state-of-the-art on the preparation of such films involves the incorporation of NPs in the thin films, and films with a multilayer structure. The carried-out investigations have been mainly conducted with the focus on preparation of antibacterial surface coatings by combining the developments in nanomaterials preparation and CAP. For instance, metal NPs with antimicrobial or bactericidal properties were incorporated or coated onto the surfaces of implants by plasma modification techniques. In addition to the use of CAP as implant coatings, CAP have been used to enhance the antibacterial properties of CuO-NPs by enhancing their biocompatibility as well as antibacterial activity by achieving thin layers of various polymers on their surface. This prevents the oxidation of metallic nanoparticles, and allows the diffusion of metal ions that will act as antimicrobials primarily with the additional dispersing effect of the plasma-polymerized surface modifications. Therefore, CAP treatment is ascribed to ease the preparation of antibacterial coatings and enhancing the impact that can be employed as preventive strategies for the bacterial infections.

Pioneering methodologies, such as nanotechnology, to control and extend antibacterial activity are essential to create new solutions and products. Passive approaches in sustained antibacterial release can be provided by simply altering the concentration, distribution, size, and/or charge of the coated antibacterial materials in addition to functional surfaces with roughness, pores or organic functional groups. However, under some circumstances the release of bactericidal content may not be aimed for, due to the adverse health effects. For instance, organic biocidal agents on material surfaces are effective means to kill surface-
attached bacteria, which can inhibit biofilm formation. Synthetic biocides have been extensively investigated in the literature, such as quaternary ammonium compounds and cationic conjugated polyelectrolytes. However, such compounds could cause adverse health effects and therefore, release of these biocidals is not desired.\[126\] Thereby, in recent years, researchers have tried to design formulations to overcome the drawbacks of synthetic biocides by providing non-releasing systems. Atar-Froyman et al. have prepared soft linings as wound dressings containing cross-linked quaternary ammonium polyethyleneimine NPs, without compromising the mechanical and biocompatible properties of the linings.\[144\] Enzymatic biocides are attractive alternatives to synthetic biocides. By considering the environmentally friendly and non-toxic properties of enzymatic biocides, researchers are keen on making use of them also as antibacterial coatings. However, these investigations have not been driven yet towards implant or prosthetic coatings with incorporated NPs.

Alternatively, active approaches by means of stimuli-responsive material coatings are considered another methodology to extend the antibacterial effect of coatings. Stimuli-responsive materials have been explored for decades in the biomedical field to serve in self-healing, as actuators, and in controlled drug release.\[108\] They are usually designed to encompass two constituents; one layer that is designed to selectively react only to one or two triggers changing the permeability of the shell. The permeability of the outer layer is determined by the balance of electrostatic interactions of surface charges that tend to expand the surface and, therefore, destroy the shell compartments resulting in interaction contribution to the surface energy that, in turn, tends to reduce the surface and lead to the release of the content.\[145\] The trigger for the container shell can be exogenous stimuli such as magnetic field, temperature, acoustic waves or light; in addition to endogenous stimuli such as enzymes, pH, or altering redox states during the disease condition.\[9,145\] In a recent report by Canaparo et al., the authors have pointed out the importance of stimuli-responsive nanocontainers that can act as antibiotic delivery systems, leading to smart therapeutic responses as antibacterial coatings; e.g. (i) controlled release of payload in the specified biological compartment and (ii) rapidly being able to act to fight the pathological occurrence.\[9\] The stimuli-responsive approaches have
been envisaged to be reversible when they are designed to act only in the case of specific stimuli. There have been promising findings on the *in vitro* antibacterial impact of stimuli-responsive antibacterial NPs.\(^{146}\)[110] In the field of antimicrobials, the integration of stimuli-responsive nanomaterials into antibacterial medical products is still at a pre-clinical or academic research level.\(^{147–150}\) Some examples of wound dressings, catheters, bone cement and cardiovascular implants containing nanosilver or silver-loaded nanocomposites as antimicrobial agent is already available, and mainly nanosilver out of all nanomaterials is in clinical use.\(^{151–153}\)

To date, the stimuli-responsive nanoparticle coatings on in-door settings and implantable devices have been applied as preventive strategies against transmission of bacterial infections. In these designs, the trigger for the antibacterial activity is expected to be non-hazardous and non-invasive. Therefore, researchers have developed antibacterial coatings for the combinatory use of non-invasive photodynamic and plasma treatments. For instance, Wong et al. designed silver nanostructure-coated TiO\(_2\) thin films that encompass a high visible-light responsive antibacterial property. In their findings, the results revealed that the silver and TiO\(_2\) composites showed a synergistic antibacterial activity under visible light illumination.\(^{154}\) Thus, a long-lasting indoor antibacterial film coating could be provided in order to eliminate the transmission of nosocomial infections. In addition, Pallavicini et al. have designed self-assembled gold nanostar (GNS) coating to be triggered by near-IR photothermal activation for biofilm eradication.\(^{155}\) The researchers pointed out the possibility of fabricating medical devices with the GNS coating to be treated *in situ* with photothermal laser treatment in case of biofilm formation, i.e. through tissues. However, photothermal treatment triggered antibacterial effects has not yet been properly ensured in order to confirm the success of the prepared coatings for clinical application. Therefore, the quest for finding suitable designs for implant coatings that can be triggered with non-invasive stimuli such as the photodynamic approach is still continuing. More recently, Bagchi et al. have designed NIR-light active ZnO-based nanohybrids and coated them onto implant-mimicking titanium, and have presented their results on the light-triggered disruption in the adherence property of matured biofilms.\(^{156}\) In their studies, ZnO-NPs was conjugated with red-light-
absorbing dye squaraine (SQ) as the photosensitizer for aPDT. The designed ZnO-SQ nanohybrid employed both the stimuli-responsive nature of NIR light sensitivity of the SQ dye and acidic pH sensitivity due to the nature of the ZnO. The authors have described the acting mode of ZnO nanoparticle as a molecular-level interaction between two moieties that have been initiated by the photoinduced excited-state electron transfer process from dye to ZnO, which improved the ROS generation capability of the hybrid material. In their clinically relevant investigations on the ZnO-SQ coated titanium sheets, 48 h incubation with S. aureus culture was carried out. There was a remarkable presence of proliferating biofilms before light treatment; however, 70 % biomass reduction could be obtained when the sheet was illuminated under red light for 1 h, suggesting disruption of biofilms due to photoinduced ROS generation by ZnO-SQ. This is foreseen as a promising strategy to be used as antibacterial coatings for superficial or dental implants.

On one hand, stimuli-responsive antibacterial nanosystems are still in a relatively early stage of development, and challenges related to their triggering reproducibility with exogenous or endogenous stimuli for in vivo antibacterial approaches are imminent. On the other hand, their implementation as antibacterial coatings can bring superior advantages especially on-site that require the precise release of certain antibacterials when autoclave or sterilization is not possible, or as prevention of any bacterial infection risks. Consequently, nanomaterial-based coatings offer a versatile solution for precise and efficient strategies to prevent bacterial infections, although to introduce functions that survive the coating manufacturing process have presented itself as a key problem. There is a need to provide solutions for manufacturing of long-standing nanostructured antibacterial coatings withstanding the full manufacturing process in industrial-scale production.

2.3. Nanomaterials for the delivery of antibacterial therapeutics

Nanocarriers as drug delivery systems are commonly employed to overcome the limitations of poor water solubility and low permeability of the drug, by delivering the drug in a controlled manner. It is well known in the drug delivery field that different strategies can be used in order to inhibit the side effects of drugs, in addition to circumventing or overcoming risks of drug resistance. More specifically from the point
of antibiotic delivery, nano-drug delivery systems can also be pivotal in elevating the efficacy and safety of antibiotic administration. Nano-antibiotic delivery systems can improve the therapeutic index of antibiotics, and nanocarriers can be designed to provide improved access to the site of interest.\textsuperscript{[159]} In this context, self-assembled structures are commonly employed to reduce the side effects and the abuse/misuse of antibiotics for those that have regulatable bacteriostatic activities.\textsuperscript{[160]} In the literature, researchers have designed a variety of highly ordered supramolecular antibacterial systems to include antibiotics, antibacterial peptides, metals, PDT and PTT agents; not only possessing unique properties, but also having extraordinary functions with the aid of different nanoparticle engineering approaches.\textsuperscript{[161]} For instance, significant efforts have been devoted to provide stimuli-responsive drug delivery nanoparticles to release their cargo “on-demand,” e.g., in the presence of a certain enzyme or upon a pH-drop\textsuperscript{[161]} to further improve localized therapeutic effects.\textsuperscript{[130]} In addition, targeted drug delivery in bacterial infections has started to be increasingly employed in light of the gained knowledge in targeted chemotherapeutic delivery in oncology. By considering the bacterial infections pathophysiological pathways’ similarity to tumor tissues, a few delivery systems have achieved bacterial targeting and on-demand release of antibiotics.\textsuperscript{[163]} For instance, researchers have designed self-assembled amphiphilic copolymer-based nanoparticles to provide targeting, pH and enzyme triggered drug delivery systems (Figure 8) in order to provide precision in bacterial species selectivity and increasing the impact of antibiotics at the site of the infection.
Figure 8. Micelle formation, bacterial targeting and drug release from Vanhyd-PECL micelles. After VAN-mediated bacterial targeting, the VAN shell is removed from micelles via the cleavage of hydrazone bonds under acidic conditions, and the PCL core is degraded by lipase overexpressed at the injection site, followed by CIP release and bacterial destruction. Reproduced with permission [163] Copyright © 2018 American Society of Chemistry.

Recently, such nanocarrier designs encompassing both targeting capability and stimuli responsiveness simultaneously has been reported to create a “domino effect” upon bacterial infection treatment. The success of the domino effect strategy on in vivo MRSA infected animal models has been demonstrated by Wu et al. employing endogenous stimulus-powered controlled drug release systems delivered in a targeted fashion. [164] As shown in Figure 9, a bacterial toxin-targeted and oxygen-triggered antibiotic release system was developed based on liposome-based nanoreactors. The antibacterial capability of this system against MRSA infection was evaluated using a mouse skin infection model. Such multimodal strategies, again familiar from the vast research within the field of nano-oncology, can be foreseen as a new concept with nanomaterials for the delivery of antibacterial therapeutics for the treatment of bacterial infections.
Evolving technologies and strategies for combating antibacterial resistance in the advent of the post-antibiotic era. Advanced Functional Materials (2020) https://doi.org/10.1002/adfm.201908783 © 2020. This manuscript version is made available under the CC-BY-NC-ND 4.0 license http://creativecommons.org/licenses/by-nc-nd/4.0/

Figure 9. Design and characterization of liposome-based nanoreactors. A) Solid phase-changing material (PCM) based nanoreactor dissolves in melted PCM at 37 °C and transforms into a transparent liquid phase for a controllable drug release. B) The scheme of endogenous stimulus-powered antibiotic release from Rifampin-CaO$_2$@PCM@Lecithin nanoreactors for bacterial infection combination therapy. Reproduced with permission from [164] Copyright © 2019, Springer Nature.

The example above showcases how nano-drug delivery systems can offer promising solutions also against multidrug-resistant bacteria. Antibiotic-resistant bacteria can be effectively treated by tagging the NPs with antibiotics to provide restoring of the ability of antibiotics to destroy the bacteria. Furthermore, NPs can direct these to the bacterium-antibiotic interface for promoting efficient interaction.[165] Combined administration of NPs with antibiotics as the treatment regimen can reduce the required amount of both antibiotics and NP, thereby reducing toxicity risks and synergistically enhancing the effects of their antimicrobial activities.[166] In addition to tagged nanocarriers, stimuli-responsive antibiotic releasing systems own the constituents to achieve stimuli-triggered response and provide synergetic effects between the
antibacterial content and exogenous stimuli. By employing these multimodal strategies, antibiotics that have been rendered ineffective can be revived and old drugs can be dressed with new action modes with the help of combinatory treatment regimes in order to provide not only a cocktail of chemo-based strategies, but also bacteria-destructive treatments.

2.3.1. Combinatory antibacterial photodynamic therapy and nanoantibiotics

The combinatory approaches of NP-based treatments and aPDT can be employed to (1) create new chemical approaches yielding effective and compatible PSs in clinical application, (2) to overcome the microbial permeability barrier, and (3) to develop unique transport systems for PSs to interact with cells. The design of NP is mainly employed to improve the delivery of PSs to the site of interest. In the recent studies of Darabpour et al., the anti-biofilm effect of aPDT with the employed PS (i.e. methylene blue) can be improved once methylene blue was encapsulated into chitosan NP. A similar strategy was also successfully presented by Huang et al., who used ruthenium nanoparticles by adding acetylcholine to its surface to increase its effect in both PDT and PTT, whereby they eliminated multi-drug resistant P. aeruginosa. Superior activity improvements by aPDT could be provided not only by encapsulating or chemical integration of PS in/onto NP, but also combinatory treatments of metal/metal oxide NP with PS as reported by Forzaneb et al. In their study, silver NP and indocyanine green (ICG) were introduced into E. faecalis bacterial solution and irradiated with a 808 nm light source, and successful 99,12% in vitro inactivity could be induced. The obtained results were ascribed as a promising approach for root canal disinfection.

Researchers have also been investigating different NPs in order to provide efficient loading of PS as cargo, which can lead to effective dosage for the aPDT. Among the designed NP, mesoporous silica nanoparticles (MSNs) has high surface-to-volume ratio and pore volume; carried by which the activity of PSs can be improved for treating pathogens such as P. aeruginosa - one of the main sources of nosocomial infections. By using the adjustable porous structure of silica as an advantage, toluidine blue was encapsulated into MSNs to provide for antibacterial and anti-biofilm efficiency on different pathogens.
Nano-strategies can offer solutions in order to overcome the disadvantages of employed PSs. For instance, the efficacy of aPDT can be improved with the phenomena of two-photon excitation (TPE) mechanism. TPE is based on the use of two photons instead of one photon, whose total energies are equal to the energy of a photon excited. TPE phenomena aids to minimize high scattering and photobleaching problems of the PS, especially in the sense of acquiring deep tissue penetration. Excitation in the long-wavelength region of NIR used to excite TPE allows deeper tissue penetration to achieve efficient PDT for deep-seated tumors in cancer therapies. Therefore, it is foreseen that similar considerations in getting benefit of PSs can be provided for deeper tissues in the treatment of infectious diseases. Coherent TPE therapy in NIR has the ability to target the area in a 3D spatiotemporal manner, due to its high resolution resulting from low scattering. By this way, tumors can both be diagnosed and treated with high accuracy.

Despite the advantages of the TPE-NIR therapy, the use of photosensitive molecules results in some drawbacks as in conventional PDT, such as low stability in blood, use of excessive doses of the drug to increase efficiency, and limited targeting ability. A combination of TPE-NIR therapy with nanomedicine can enhance the efficiency of the PDT along with the increased half-life of the PS. The use of NP carriers enables stabilization of the PS in the bloodstream and increases of half-life, both of which lead effective treatment. Among the various types of nanoparticles, MSNs have attracted interest for use in TPE-NIR therapy. MSNs are used for many purposes from bioimaging, cancer therapy, antibacterial treatment, drug delivery to vaccine delivery. High surface area and large pore volume enable loading of various active agents, PSs or fluorescent dyes, into MSNs for multimodal and prospectively theranostic applications. Because of these properties, MSNs are promising candidates also for nanoparticle-mediated TPE-NIR therapy. Having a hydrophilic surface makes the MSNs suitable for the distribution in aqueous solutions that can increase solubility and half-life of the PS. Incorporation of the PS into MSN can also inhibit undesired UV or visible light excitation. To date, such parameters as surface functionalization, size, and shape have shown to be effective for maximizing the cellular internalization of MSNs. By taking this information into account, PS and/or
fluorescent dye-loaded MSNs can be functionalized for site-specific delivery into the target area and to increase cellular uptake through enhanced cell-nanoparticle interactions. As an example of bioimaging applications through MSN-mediated TPE-NIR therapy, Geng et al.\cite{175} carried out a study to achieve real-time brain vascular imaging in mice. A common fluorescent dye, poly(9,9-dihexylfluorene-alt-2,1,3-benzothiadiazole) (PFBT) was incorporated into the micelle-MSN composite nanoparticles. After administration of the synthesized nanoparticles, in vivo brain blood vessel visualization was performed upon excitation at 800 nm. As a result of the study, fluorescent properties of the PFBT were enhanced with the use of the MSNs while an imaging probe with high stability and biocompatibility was developed. Apart from MSNs, organically modified silica (ORMOSIL) nanoparticles have also been put forward as promising candidates for in vivo bioimaging. ORMOSIL nanoparticles enable encapsulation of hydrophilic or hydrophobic fluorescent dyes, which in turn can result in good fluorescence quantum yield depending on the selection of the dye. Considering that ORMOSIL nanoparticles are transparent to visible and NIR light, Qian et al. carried out a study related to in vivo imaging and two-photon PDT by using photosensitizer PpIX and NIR fluorophore IR-820.\cite{176} PpIX doped silica nanoparticles were shown to effectively kill HeLa cells through TPE-PDT. During in vivo imaging studies, IR-820 doped silica nanoparticles effectively visualized target regions with different depth profiles. NIR imaging was also used for sentinel lymph node and tumor imaging at certain time intervals, which showed the success of the developed probes for both TPE-NIR and PDT. Croissant et al. used disulfide-gated, multifunctionalized MSNs for TPE imaging and drug release.\cite{177} A disulfide bridge was used to prevent the early release of the loaded two-photon electron donor and the anticancer agent, doxorubicin (DOX). The “nanogates” were activated by laser light treatment at 760 nm to release the electron donor and DOX in order to achieve two-photon cancer therapy. In vitro studies showed that multifunctional MSNs were successfully taken up by the cells through endocytosis and achieved the desired release for realizing a theranostic approach.\cite{145} These successful studies together with different nanoparticle systems and TPE on cancerous cells for diagnostic and therapeutic purposes open a new field to deal with infectious diseases by increasing the efficiency of aPDT. As mentioned above, in order to provide effective antibacterial
impact with PDT, different challenges due to limited penetration of light into the deeper tissues has to be overcome.

Most of the PSs used in PDT are hydrophobic and have limited solubility in aqueous environments. Thus, many problems related to the hydrophobic nature of the PSs are encountered during PDT applications.\(^{[178]}\) Self-assembly of peptides and proteins is one of the most useful and easy methods to overcome these PS-dependent negative sides of PDT. Furthermore, since the building block of these assemblies is composed of natural amino acids, they have excellent biocompatibility, biodegradable properties and high potential for being used in phototherapy approaches since they can be produced flexibly under the desired conditions. It increases the efficiency of the photosensitive agents, thus increases the effectiveness of phototherapy in antibacterial applications.\(^{[179]}\) Liu et al. has developed a NP system that can perform bacterial imaging, on-demand targeting and antibacterial therapy on a single NP platform.\(^{[180]}\) This strategy aids the accessing of PS to the low-pH infected site without early release of it during therapy. The effect of aPDT was investigated by developing a porphyrin-nitric oxide NP system with non-covalent self-assembly of porphyrin, which is a very commonly assessed PS on *E. coli* and *S. aureus* cells.\(^{[181]}\) The results showed that the NP system increased the photodynamic effect not only through ROS but also with the antibacterial activity of nitric oxide, by creating high toxicity in bacterial cells. In another study, Jia et al. aimed to load PS molecules on the bacterial surface by attaching protoporphyrin photosensitizer to cholesterol modified polyethylene glycol polymer NPs.\(^{[182]}\) It was found that the cholesterol part in the NP system facilitates the high binding of PS to the bacterial surface and provides successful drug delivery. Thus, 99.99% photodynamic killing was achieved on Gram-negative *E. Coli* and Gram-positive *S. aureus* bacterial species.\(^{[182]}\)

In summary, while PDT offers an effective measure as antibacterial treatment, its effectiveness can be enhanced through numerous NP designs. The main benefits of using nanomaterials in the field of aPDT are increasing efficiency, lowering the PS concentration, and increasing the delivery to the lesion site.

2.3.2. Nanomaterials concomitant to cold atmospheric plasma treatments
CAP and CAP-treated liquids provide strong antimicrobial activity. In addition to this, their combination with various antimicrobial agents and antibiotics show synergistic antimicrobial activity even on multidrug-resistant (MDR) bacteria. Furthermore, combinatory use of CAP and antibiotics for the treatment of MDR bacteria leads to lesser minimum inhibitory concentration (MIC) levels for various classes of antibiotics. Thus, combination of CAP with antimicrobials and antibiotics seems to be a viable strategy to combat infections caused by MDR pathogens. Similar to combination of CAP and antimicrobials, also combination of CAP with nanoparticles has been attracting the attention of the scientific community for antimicrobial applications; especially to provide highly localized target-specific treatment and delivery to specific diseased areas. In the case of nanoparticles, plasma could aid in antibacterial nanocapsules fabrication with enclosed plasma RONS, and a concomitant plasma treatment of diseased tissue can temporarily open pores to allow for easier access of inherent antibacterial NPs and antibiotic carriers.

Both plasma medicine and nanotechnology are rapidly growing fields with promising applications that became a part of clinical practice. As both fields present excitingly remarkable achievements in terms of antimicrobial effects, they can be used in concomitant treatments in order to combine their advantageous properties to provide antibacterial impacts. The possible acting of CAP and NPs can meet the challenges of this battle. The first one is the production and synthesis of antibacterial nanoparticles that are known as plasma-nanoscience. In general, plasma nanoscience focuses on the specific roles and benefits of the ionized gas (plasma) environments in assembling, processing, and controlling nanoscale (including biological) objects. In practice, plasma with the high concentrations of ROS can directly reduce metal ions in solutions to form metal nanoparticles that remove the need for additional reducing agent, and making plasma a green technology for nanoparticle fabrication. In terms of nanoparticle production by CAP treatment, CAP generated RNOS could be encapsulated in nanomaterials that will increase the lifetimes and penetration capabilities of RONS for applications. The encapsulation of RNOS generating sources can also be encapsulated in particular NP designs, whose antibacterial activities can be CAP coded. In addition to these practices in creating plasma-nanoparticle synergy as an antibacterial strategy, plasma modification of NPs with plasma
polymerization approach is employed as a promising strategy to provide non-agglomerated NPs that can provide biocompatible and more effective antibacterial treatments compared to their non-modified counterparts. A second strategy where CAP and nanomaterials can meet is their combinatory or synergistic use as presented in Figure 10. By means of this approach, CAP is capable to enhance the penetration of nanomaterials to cells by cell membrane permeabilization, and their combination could exert stronger antimicrobial effect as both of them have their own antimicrobial effect. Reactivity, selectivity, toxicity and penetration capabilities of CAP and nanoparticles are the factors that are needed to be considered for their use in biomedical applications including antimicrobial applications, and concomitant CAP with nanoparticles are foreseen to lead to more effective and safe use in antibacterial treatments.

In the literature, researchers have already reported on synergistic antimicrobial effects of CAP and NPs. For instance, utilization of 30 nm AuNPs with CAP has increased the inactivation of Streptococcus mutans on tooth to 5-log, compared to 3-log reduction achieved by only CAP treatment. The increase on the inactivation of the S. mutans by combination of CAP and AuNP has been supported with transmission electron microscopy (TEM), which revealed cell wall damage by CAP treatment, and further cellular damage that was accompanied with loss of intracellular structures by AuNPs. Similarly, combinatory application of CAP and
NPs led to enhanced antifungal effect. A combination of CAP with Ag nanoparticles significantly reduced the MIC50 and MIC100 values of five different dermatophyte fungi with varying tolerance levels. Furthermore, combinatory use of CAP and AgNPs also showed significant healing suppression of disease symptoms on dermatophyte namely, *Microsporum canis*, inoculated guinea pigs. While fluconazole (152 μg/ml) (a conventional antifungal), AgNPs (13 μg/ml) and CAP led to 79.2%, 70.8%, and 42% healing after inoculation of *Microsporum canis*, respectively, the healing for the combination of CAP and Ag nanoparticles was determined as 91.7% for the same period. The reduction of MIC values of those dermatophytes by combination of CAP and AgNPs was shown to be due to longer lifetime of CAP generated reactive species as they were carried by NPs and improved penetration of NPs to fungal cells through the cracks on the cell that were caused by CAP itself.\[190\] Similarly, combinatory of CAP and AgNPs that were encapsulated in polymersomes showed promising bacteriostatic or bactericidal effect on an MDR *E. coli*. The growth of *E. coli* was delayed at 25 to 75 μg/ml AgNP concentration, while complete inactivation of *E. coli* was achieved at 100 μg/ml AgNP when combined with CAP.

The concomitant use of CAP with antibacterial nanostructured materials has also been explored. In the study of Ze et al., authors have investigated the improved antibacterial activity of nanostructured thin graphene oxide (GO) layer.\[142\] The combinatory use of thin GO layer with CAP via treatment of GO with inductively coupled radio frequency driven H2 CAP was conducted, whereby the reduction of the GO layer led to significant growth inhibition of *E. coli*. When the untreated GO layer could not cause any significant antimicrobial effect even at the remarkably long contact time, the plasma-treated GO layer was capable to exert significant antimicrobial effect at 30 minutes of contact, and complete inactivation when the contact time was extended to 60 minutes. The significant improvement of the antimicrobial effect of GO layer by the treatment of non-thermal H2 CAP treatment was attributed to the severe cell membrane damage due to reduction on the lateral size of the GO layer, which was determined to be around 0.5 μm with a thickness of 1 nm after non-thermal H2 plasma treatment. The mainstream of the carried out research on combining CAP and NP usually focuses on bacterial growth inhibition of planktonic growth mode of bacterial species.
However, it is well known that CAP has also shown tremendous effect on eradication and prevention of bacterial biofilms.\textsuperscript{[191]} While CAP and NP combination is attributed as an attractive strategy inefficient inactivation of MDR pathogens, similar strategies have not been tested for the elimination of bacterial biofilms.\textsuperscript{[192]} The field is lack of findings on combinatory CAP and NPs to fight against biofilm-associated bacterial infections. When the strategies for the prevention/destruction of biofilms is considered, the first principal strategy is inhibiting the adhesion of bacteria to surfaces, thereby the chances of biofilm formation can be eliminated.\textsuperscript{[193]} The second strategy is aimed at the destruction of biofilm matrix during the maturation process\textsuperscript{[194]} and the last strategy is the signal interference approach, which involves the inhibition of quorum sensing (QS).

During the combinatory application of CAP and nanomaterials, the RONS induced by plasma can be encapsulated inside the materials; hence, their lifetime and travel distance can be increased and enhance the effect. As another strategy, CAP can aid to opening or enlargement of the cell membranes therefore, cell permeability might increase and lead to a better NP internalization into the bacteria. Further studies are required to understand the nature and extending the use of plasma and nanomaterials combination in order to provide biological impact. In addition, the low selectivity property of non-thermal plasma can be compensated by nanomaterials, by modulating the NP with specific targets.

3. Conclusion

The current global threat of antibacterial resistance has spurred alternative developments to drug therapies, given that the development of new antibiotic drug compounds cannot keep up with the rate of resistance evolution among pathogens. Novel technologies seem to be promising complementary strategies to account for the lack of new antibiotic drugs. Concomitant use of nanotechnology, phototherapies, and plasma medicine in different ways as have been outlined above, may provide for synergistic effects not achievable by one method alone. In this progress report, we have specifically aimed at presenting recent
materials-driven approaches in enhancing the efficacy of these novel technologies in the combating of bacterial infections.

Nanotechnology in itself implies the utilization of nanomaterials for specific applications, in this case fighting microbes such as bacteria. Several inorganic nanoparticles possess inherent antibacterial activities, and especially silver NPs have been used since ancient times. To make inorganic NPs more functional and responsive to its environment, they can be combined with organic constructs to form hybrid (nano)materials. The multifaceted tasks such constructs can perform in an antibacterial context has been exemplified above, but on the downside it needs to be kept in mind that the more complex the design, the more difficult and costly the clinical translation will be. Hybrid nanomaterial constructs are nevertheless not only limited to therapeutic interventions, but can be highly useful in other aspects of fighting bacteria e.g. in antibacterial coatings, diagnostic kits, biosensor systems, and other preventive strategies against bacterial infections as well as constituting vital components for bacterial detection and identification.

When antibacterial PDT (aPDT) is compared with conventional antibacterial strategies, it has many advantages such as being less invasive, safer and more effective; and the more important aspect is that developing antibacterial resistance against the mechanism of PDT is still quite impossible. However, many applications of PDT have resulted in discomfort because of pain, burn sensation and the need to keep the patient away from any light source. Therefore, there is still a need for different strategies to be implemented into PDT to decrease or eliminate discomfort of the patient. One of these upcoming and promising ways to decrease this is the use of different nanoparticle systems. They offer new agents or carrier systems for the transfer of photosensitzers, specifically targeted to the application area, to be accumulated in specific cells and rapidly removed from other parts of the tissue. Consequently, integrating nanotechnology with PDT can provide the opportunity to improve the effectiveness of PDT and decrease the adverse effects on patients in the near future.

From the aspect of combinatory approaches of nanotechnology with CAP, there is still need for a clear understanding of the CAP antibacterial action mechanism in order to prepare CAP responsive antibacterial
nanomaterial designs to have on-demand action, either for prevention or therapeutic approaches. Additionally, by acquiring benefit of reversible impact of the cold plasma technology on different tissues, CAP can be used to improve the delivery of antibacterial nanoparticles e.g. through the skin for enhanced transdermal drug delivery. Despite the strong and broad-spectrum antimicrobial effects of the cold plasma technology, it could be applied from the surface where the infection develops. However, plasma-assisted transdermal drug delivery could make cold plasma technology useable to enhance the systemic administration of antimicrobial agents including antimicrobial nanoparticles. Thus, in addition to combinatory use of cold plasma with nanoparticles to combat infections, plasma could also be considered a novel method for enhanced delivery of nanoparticles through the skin for their systemic administration. These techniques can further be utilized also for the diagnosis and prevention of infectious diseases, thus constituting a very versatile platform in the combating of MDR bacteria and bacterial biofilms.

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