Chapter 3

Nano-Antimicrobial Solutions Using Synthetic-Natural Hybrid Designs

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Abstract

Nanotechnology potential in antimicrobial therapy is increasingly demonstrated by various data. Results reveal antibacterial properties, comparable to that of conventional antibiotics. Working on parallel experiments, researchers continue to bring evidence demonstrating age-old-recognized antibacterial properties of various natural components of plant and animal origin. Later years brought an increasing trend for combining synthetic and natural composition in new constructs. The tendency aims to bring more on different essential aspects, such as active substance release, improvement of antibacterial effect, and up-regulation of the mechanisms at the structure-cell interface. Present chapter structures the up-to-date achievements in the field, including the concept of design, biological effects, benefits, mechanisms, and limitations of the field. Also, expected future research directions are to be discussed.

Keywords: antimicrobial, synthetic, natural, nanotechnology, antibacterial

1. Introduction

Microbial infections represent a major health problem, being responsible for more than 16 million cases of pathology-related death cases per year [1]. The impact is increased by the appearance of multidrug-resistant bacteria, a mounting tendency, responsible for both acute

and chronic forms of clinical presentations of such infections. The need for urgent generation of new, valid therapy solutions, capable of eluding the resistance mechanisms, has been increasingly high during late years. Nanotechnology potential in antimicrobial therapy is increasingly demonstrated by various data. Nanoparticles such as zinc oxide, silver, aluminum oxide, iron oxide, copper, titanium dioxide, and silicon dioxide have been successfully tested by various research groups [1]. Results reveal antibacterial properties, as demonstrated by testing strains of *Escherichia coli* [2], *Staphylococcus aureus* [3], *Staphylococcus epidermidis* [4], or *Pseudomonas aeruginosa* [5]. Working on parallel experiments, researchers continue to bring evidence demonstrating age-old recognized antibacterial properties of various natural components of plant and animal origin. The presence of phenols and phenolic acids [6], quinones [7], flavonoids [8], tannins [9], terpenoids [10], alkaloids [11], lectins, and other polypeptides [12, 13] in the composition provides bactericidal or bacteriostatic effect by activating various biological mechanisms. Present chapter summarizes the most recent achievements in new designs of therapeutic solutions involving both natural components as well as laboratory processing and/or synthetic components. Also, some of the most common antimicrobial mechanisms of those structures are to be analyzed.

2. Green synthesis of nanoparticles exhibiting antimicrobial role

The idea of nanoparticle synthesis using green technology represents one of the first, beginning trends in joining the two different domains: nanotechnology and natural extract chemistry. Although the final composition of the nanoparticles designed this way does not necessarily include high concentrations of natural extracts, the concept of green design aims to diminish the risk of possible chemical traces resulting from nanoparticle synthesis in the final product. Reducing or stabilizing agents could be good examples of such traces. In the new green synthesis concept, any traces, if present, would be a part of a natural compound with rather beneficial than dangerous effects. One such report is the synthesis of silver nanoparticles using *Acorous calamus* rhizome extract. The extract was prepared starting from *Acorous calamus* rhizome powder, using a mixing (100 mL soluble distilled water) technique, associated with heating (60°C, 10 min) and filtering procedures. Manufacturing of nanoparticles was further performed using a 5:1 ratio mixing of aqueous $AgNO_3$ solution and natural extract, respectively, followed by room temperature, 24 h of incubation. Centrifugation (18,000 rpm), repetitive washing followed by room temperature, and drying were used by researchers to collect the nanoparticles. Complete characterization including spectral (UV-Vis, SEM/EDX, FTIR) techniques, hydrodynamic measurements (DLS) as well as simultaneous application of thermogravimetric and differential scanning calorimetric techniques (TGA-DSC analysis) certified the synthesis of nanoparticles. The manufactured nanostructures have revealed strong antibacterial effect against *Bacillus subtilis*, *Staphylococcus aureus* as well as *Bacillus cereus*. Disk diffusion technique revealed 1.5, 1.7, and 1.6 cm if inhibition zone, compared to the streptomycin standard responsible for 3.4, 3.1, and 2.6 cm of inhibition performed against streptomycin control. Similarly, growth kinetic studies analyzing the effect of a 40 μg/mL concentration of synthetized nanoparticles on *Escherichia coli* showed significant inhibition within the log phase (active phase) of bacterial

growth, a demonstration of antibacterial activity [14]. Similar results were reported by other groups. One such research team used *Boerhavia diffusa* plant extract as a reducing agent and tested the newly synthetized silver nanoparticles for their antibacterial role. The whole-plant extract of *Boerhavia diffusa* involved collection, washing of plant, drying, followed by Soxhlet apparatus-based extraction and power formulation by reducing under pressure. Dilution of the extract (500 mg in 100 mL distilled water) and mixing with $AgNO_3$ solution (10 mL prepared extract solution: 90 mL 0.1 M $AgNO_3$) followed by heating, (100°C, continuous stirring, 15 min), discarding of supernatant, collecting, and drying of sediment were used for the synthesis of the nanoparticles. SDR, SEM, TEM, as well as UV-VIS analyzes demonstrated the formation of silver nanoparticles. Three selected fish pathogen strains were used, namely *Aeromonas hydrophila*, *Flavobacterium branchiophilum*, and *Pseudomonas fluorescens* and the determination of minimum inhibitory concentration was performed. The MIC values for *Flavobacterium branchiophilum*, *Aeromonas hydrophila*, and *Pseudomonas fluorescens* were 3.12, 25, and 50 μg/mL, respectively. Although values differed from one bacterial strain to another, the designed silver nanoparticles revealed similar diameter of inhibition zone with that of Rifampicin for concentration values of 50 μg/mL (15, 14, 12 mm, respectively, for *Flavobacterium branchiophilum*, *Aeromonas hydrophila*, *Pseudomonas fluorescens*). [15]. Similarly, callus extracts of *Sesuvium portulacastrum* L. from tissue cultures were successfully used for the generation of silver nanoparticles. Preparation of callus was performed following a previously reported technique [16]. For the synthesis of silver nanoparticles, callus extraction steps (grinding of 20 g fresh callus, boiling for 5 min, centrifugation of 3000 rpm), mixing with $AgNO_3$ solution, incubation (dark), and stabilization with polyvinyl alcohol were carried out. The antibacterial activity was found to be efficient as demonstrated by clinical microbial strain testing with inhibition zones ranging from 11 to 17 mm (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Listeria monocytogenes*, and *Klebsiella pneumoniae*) [17]. *E. coli*-directed antimicrobial activity was also documented by disk diffusion method in the case of a carob leaf extract-mediated synthesis of silver nanoparticles. Extract was performed using a consecutive step procedure including washing, sun-drying, cutting followed by boiling in distilled water, filter separation, and centrifugation (1200 rpm, 5 min) for heavy metal removing. Synthesis of nanoparticles was carried out by mixing 5 mL aqueous extract with 100 mL of 1×10^{-3} M $AgNO_3$ solution. Several techniques were involved in characterization of nanomaterial UV-VIS and FTIR spectral techniques, x-ray diffractometry, and SEM, all of which demonstrated a fast and efficient formation of silver nanoparticles. The effect has been reported to be superior to standard antibiotic, with a minimum inhibitory concentration for silver nanoparticles of 0.5 μg/L, while the standard antibiotic calculated value was 0.6 mg/L [18]. Moreover, unique designs based on silver nanoparticles, such as silk fibroin-silver nanoparticle composites, have also been reported. The natural polymer *Bombyx mori* was used as a scaffold for synthesis of silver nanoparticles in situ, under the effect of light. The synthesis included preparation of silk-fibroin solution involving degumming steps (0.5 wt% $NaHCO_3$, 100°C, 30 min), dissolving step (9.3 mol-L LiBr solution, 60°C), dialyze step, centrifugation (6000 rpm, 5 min), and collection of supernatant. Next, composite synthesis was carried out, including mixing of $AgNO_3$ powders (5–8 mg) and 1 wt% silk-fibroin solution (5 mL), followed by UV light exposure and incubation (room temperature, 24 h). The construct demonstrated biofilm-destructive properties as well as

direct antibacterial effects against methicillin-resistant *Staphylococcus aureus*. The minimal inhibitory concentration was reported to be 19.2 mg/L for silver nanoparticles within the composite material [19].

However, not only silver nanoparticles have been reported to be successfully synthetized using green technologies. One report provided evidence of triangular gold nanoparticles synthesis by using extract of *Aloe vera* plant. 6 mL of 0–3 M aqueous $HAuCl_4$ solution were mixed with distinct volumes (0.5–4 mL) of *Aloe vera* extract (obtained by butting and boiling procedures), and volume of each sample was completed up to 10 mL. The authors provide evidence of possible modulatory effect of the *Aloe vera* concentration on optical and morphological properties of gold nanoparticles. Addition of *Aloe vera* (in different amounts) can vary the size of gold nanoparticles from 50 to 350 nm. Also, possible shapes of nanoparticles include spherical, triangular, and hexagonal or rod-like patterns. The team demonstrates strong near-infrared absorbance, suggesting a good potential for hyperthermia-modulated applications, such as anticancer or antimicrobial effects [20]. Similarly, gold nanoparticles were published to have been synthetized using *Memecylon edule* leaf extract. The experiment passed through a first step of *Memecylon edule* extract, using washing, drying under dark, cutting into smaller parts, powdering (mixer), boiling (20 g powder, 100 mL water, 5 min), incubation (dark, 30°C), and filtering. In a consecutive step, a bioreduction process was carried out involving aqueous leaf extract in different concentrations (15, 10, and 5 mL, respectively) and $HAuCl_4$ solutions (1 mM, 10 mL). The gold nanoparticles presented a plate-like morphology and various shapes: triangular, spherical, tetraedrical. The dimension ranges between 20 and 35 mm. Each shape was demonstrated to induce distinct optical properties, with decisive importance on the future antimicrobial plasmonic resonance-based applications [21]. Recently, the diversity of structures and concepts of materials have been increasing. Nanosize crystals of ZnO have been manufactured using *Nephelium lappaceum* L. peel extracts. Procedures of natural extract preparation involved washing and cutting of peels, followed by oven-drying, (50°C), boiling (3 g dried rambutan, 1:2 ratio of ethanol:water for 10 min), and filtering. The technology, involving zinc-ellagate complex formation resulted in coating of cotton fabric with ZnO nanocrystals. Synthesis of ZnO nanocrystals was carried out by slow addition of 10 mL of rambutan peel extract in 50 mL of 0.1 M of $Zn(NO_3)_2.6H_2O$ sol (stirring, 80°C, 2 h), followed by several centrifugation steps, oven-drying (40°C, 8 h), and calculation (muffle furnace, 450°C). Material has been added with ZnO nanocrystals (12 × 12 cm dimension, 2% ZnO, 1% citric acid solution, 5 min,) further processed using the padding mangle run (15 m/min, 15 kgf/cm^2), and was subject to thermal drying (3 min, 140°C). Removal of unbound crystals, and soap traces and final drying ended the experimental protocol. Such material demonstrated both Gram-negative (*E. coli*) as well as Gram-positive (*S. aureus*) bacteria. According to disk diffusion results, the largest inhibition area corresponded to *S. aureus* (23 mm in diameter), while *E. coli* revealed 18.5 mm diameter inhibition [22]. Similar cotton fibers loaded with silver nanoparticles were designed by using natural tree leaves *Ficus benghalensis* and *Eucalyptus citriodora*. The extract preparation protocol included intense washing (15 min, 15°C), drying, preparation of leaf broth solution (100 mL water with 2, 4, or 6 g of leaves), heating of solution (100°C), extraction (30 min), and filtering. Synthesis of nanoparticles was performed by mixing of natural extract (2, 4, or 6 g in 5 mL) with 1 mM aqueous $AgNO_3$ solution (1:1 volume ratio). Immersion of

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cotton fibers in the Mixt solution (shaker, 1000 rpm, room temperature, 24 h) and drying ended the protocol. The authors have demonstrated efficient antimicrobial effect against *E. coli* in case of 2% leaf extract, even after repetitive water washing of treated fabric at different time intervals (5, 10, 15, 30 min, 1, 3, 5, 10, 15, and 24 h) [23]. Also, recently, a new waterborne paint additive with antimicrobial properties has been reported. The authors reported the generation of zinc oxide nanoparticles (partially covered with silver) by means of flame spray pyrolysis, followed by powder dispersion through stirring and sonication. Characterization of obtained product included specific surface area assessment, transmission electron microscopy analyzes, x-ray fluorescence testing, and dynamic light scattering measurements, demonstrating the structure, conformation, and dimensions of nanoparticles included in the product. Testing of antimicrobial effect was performed using a standardized method (ISO 22196:2011, modeled by Japanese standard JIS Z 2801:2000). In brief, survival counts were estimated after spreading of different concentrations of ZnO-Ag nanopowder (0.1, 0.2, 0.3, and 0.4%) and ZnO nanopowder on solidified plates containing 10^9 colony forming units of each of the microorganism (tryptone soy agar, 0.6% (W/v) yeast extract, nutrient agar). Results showed significant antimicrobial effect against *Staphylococcus aureus, Salmonella* spp., *Listeria monocytogenes, Bacillus subtilis,* and *Pseudomonas* spp. with an inhibition zone of >5 mm [24].

A special class of green synthesis designs involves the use of alternative sources for reduction and stabilizing agents, apart from plant or animal origin. Using bacterial, actinomycete, yeast, or fungal strains for providing the necessary material for green synthesis is more recently an interesting technological solution. The use of *Candida guilliermondii* was reported recently for silver as well as for gold nanoparticle production. The nanoparticles demonstrated efficiency against *Staphylococcus aureus*, unlike the chemically synthetized silver and gold nanoparticles who demonstrated no antimicrobial effect. The results demonstrate the enhancing of antimicrobial properties due to green synthesis protocol [25]. Intracellular synthesis of gold nanoparticles by using a special strain of *Rhodococcus* species was reported. Experimental protocol included isolation of *Rhodococcus* sp., maintenance (potato-dextrose agar slants), monthly subculturing, and preservation. Mycelia were produced by growing of actinomycete in MGYP medium, separation (centrifugation 200 rpm, 27°C, 96 h), and washing. Gold nanoparticles were isolated by resuspension of mycelia in aqueous $HAuCl_4$ solution (10^{-3} M). Successful biotransformation was certified by UV-VIS spectra, TEM, and XRD analyzes [26]. Also, *Bacillus licheniformis* has been successfully used for synthesis of silver nanocrystals. Isolation of bacteria (collection, incubation 37°C, 48 h) and characterization of isolates were followed by addition of $AgNO_3$ (1 mM) solution to 2 g wet biomass previously inoculated with bacterial isolate [27]. For another report, the synthesis was carried out by using fungal strains, such as *Fusarium acuminatum*. After isolation from infected ginger and characterization of extract, fabrication of biomass was carried out (incubation of fungal culture at 28°C in potato sucrose broth, inoculation onto flasks, agitation, and filtration). Synthesis was carried out by mixing $AgNO_3$ solution with the filtrate for a final concentration of 1 mM, followed by 2 h incubation. Such nanoconstructs, resulted from mycosynthesis and validated by UV-VIS and TEM analysis, demonstrated efficiency against *S. aureus* (17 mm inhibition), *S. epidermidis*, *S. typhi*, or *E. coli* (10 mm) in the exact mentioned order (from highest to lowest efficiency) [28].

3. Nanoencapsulation and microencapsulation of natural compounds designed for antibacterial applications

3.1. Cyclodextrin encapsulation

Increasing the efficiency of natural compounds as well as diminishing their drawbacks, such as limited bioavailability or excessive rate of release, has been one major and constant research topics during late years. Several practical approaches have been designed. Polymer-based nanoparticles as well as naturally derived nanocarriers were the most common experimental trends [29]. Phenolic compounds as well as the specific component piperine are known to be present in black pepper oleoresin. Researchers have started to improve their biological interaction properties by approaching the encapsulation in cyclodextrins. Although the capsules are relying on a natural-based material, their laboratory processing and characterizing the newly designed construct represented an important research step. Testing data also revealed not only higher antioxidant activity for the encapsulated extract, but also more efficient antibacterial effect as compared to nonencapsulated compound. Data revealed that lower concentrations are needed for inhibiting the growth of the Salmonella strain used for evaluation and demonstrated that designed formulation is able to improve the antimicrobial effect of the natural extract [30]. β-Cyclodextrin encapsulation has also been selected by Mourtzinos et al. for optimization of olive leaf natural extract properties. The active component, oleuropein has been already demonstrated to exert anticancer [31] effects, inhibitory efficiency against certain human pathogens such as Mycoplasma [32], as well as to provide antioxidant protection [33], and the obtained formulation offered protection for the natural extract toward better biological effect. Similarly, Dima et al. used an extract coming from *Coriandrum sativum* L. seeds and structured a formulation by β-cyclodextrin encapsulation. Testing revealed an intense inhibition of 2,2-diphenyl-1-picrylhydrazyl radicals for 30 μg/mL. The newly designed capsular formulation has proven to have important stronger antioxidant activity as compared to widely accepted standards (ascorbic acid, butylated hydroxytoluene). Also, antimicrobial and antifungal activities have been reported [34].

3.2. Complex coacervation

A distinct attempt of providing improved properties for natural extracts by hybrid processing was involving complex coacervation. The extract used was that of propolis, already known as a natural-source food additive. Isolated pectin and soy protein were used as encapsulation material. Although the compounds used in encapsulation were of natural origin, isolation of compounds and the complex coacervation protocol represented a step forward in improving the properties of nanomaterials by encapsulation. The authors have demonstrated the technology to generate a stable, alcohol-free agent in a powder formulation that elicits controlled release properties, but also demonstrated antimicrobial activity against *Staphylococcus aureus* [35].

3.3. Polymer-based encapsulation and liposomes

However, most researchers have focused toward synthetic, polymer-based systems as well as liposomes.

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The need for packaging food using materials with antibacterial properties motivated the work of a research team who designed nanocapsules with cinnamaldehyde. The capsules were designed as lipid bilayers of polydiacetylene-N-hydroxysuccinimide (PDA-NHS) nanoliposomes. Immobilization on glass slide was further performed and this type of product demonstrated significant antibacterial activity against *E. coli* as well as *Bacillus cereus* in (2.56 log10 and 1.59 log10 CFU/mL, respectively; reduction in 48 h) [36]. The efficacy of liposomes containing cinnamon natural extracts against methicillin-resistant *Staphylococcus aureus* (MRSA) was also demonstrated in a recent article and was appreciated as satisfactory by the team. As colony forming unit determination reveals, such formulation could offer high efficiency against MRSA biofilms on various classes of substrates, from steel, nonwoven fabrics, gauze, and up to nylon membranes. The formulation was demonstrated to augment stability of antibacterial effect and to prolong the period of action [37]. Similarly, Fennel extract was encapsulated by another center, in the attempt to create food additives able to exert antimicrobial role on the fish meat (carp species). The extract demonstrated antioxidant effects, as well an antibacterial effect as revealed by the microbial count. The efficacy of the liposome-encapsulated form proved superior in terms of oxidative deterioration to tissues and reducing of microbial colonization. The formulation also provided extended shelf life following treatment of carp fillet [38]. Another recent report also demonstrated superior antioxidant and antimicrobial properties of Thymus species extracts. The team obtained an enriched antioxidant activity and antimicrobial effect of the liposomes containing extracts coming from the four selected species of Thymus as compared to the extract alone [39]. Similar thyme extract was encapsulated into liposomes by researchers and the effect on microbial colonization and oxidative injury on silver carp was analyzed compared to controls using a 15 day monitorization period. The growth inhibition of *E. coli* O_{15}:H_7 was demonstrated, and the total bacterial estimation in the food had proved superior in the encapsulated formulation as compared to natural extract, therefore presenting the designed liposomes suitable for carp meat food additives [40]. Driven conclusions were equally sustained by a distinct research group, who finds the phytosomes as suitable for drug and food applications, their stability, physicochemical properties, and antibacterial efficiency being dependent of specific method of synthesis. The liposomal encapsulation is considered as preserving the activity of bioactive components as compared to water solution, this particularity being caused by the elevated water solubility and reduced lipid solubility [41].

A more extensive study tested various encapsulation designs for active components such as lysozyme, nisin as well as various herbs and spice extracts, including liposomal, chitosan as well as polysaccharide encapsulation. The advantage of liposomal formulation could come from their higher stability compared to chitosan encapsulation. Antimicrobial activity against both positive and negative of Gram bacteria was efficient and stable for a minimum of 1 month. Due to the controlled release possibility derived from the formulation concept, the authors indicate a large potential for applications under hydrogel form with embedded capsules containing natural extracts [42].

Recently, the synthesis protocols became more oriented toward complex structures, such as polymer-lipid nanoparticles. One of the most robust designs is represented by a core-shell concept, presenting a polymeric core, a lipid shell with embedded active substance, and

protected by polyethylene-glycol moieties for immunoreactivity reduction [43]. The advantages of such structures come from increased stability, morphological and structural integrity, low risk of damage during storage, controlled release features, elevated biocompatibility, and bioavailability. Both the polymeric and the lipidic component can be built using not only artificial, but also using natural sources, such as chitosan or natural fatty acids and represent the next generation of materials directed toward antimicrobial applications [44].

4. Nanoparticles functionalized with natural biomolecules

Not all research groups have followed the encapsulation trend. A part of the research teams have focused on direct attachment of biologically active, natural origin molecules onto the surface of metal nanoparticles. One such design was the synthesis of catechin-Cu nanoparticles. By joining two elements with already known antibacterial effect, the newly formed compound was reported to induce a 3 h-death rate of up to 90 and 85% of *S. aureus* and *E. coli*, respectively, as assessed by means of the live/dead bacterial viability kit by the authors [45]. Similarly, iron oxide nanoparticles were functionalized with natural source gallic acid. The resulting construct was demonstrating significant antibacterial effect against *E. coli*, *S. aureus*, and *B. subtilis*, comparable with that exerted by ampicillin or streptomycin [46]. Also, chitosan, a natural polysaccharide, was demonstrated as presenting improved efficiency when binded to copper or zinc nanoparticles, and the effect has been published to be proportional to the level of zeta potential [47].

Silver nanoparticles were also reported to have been successfully functionalized with glucosamine, a natural sugar. The newly constructed compound presented high antimicrobial efficiency. Both *Klebsiella pneumoniae* and *Bacillus cereus* were more sensitive to the functionalized as compared to pristine AgNps, as demonstrated by minimum inhibitory concentration determination [48]. Research has been advancing toward ore and more complex designs. In another publication, a crosslinked chitosan-coated Ag-loaded nano-SiO_2 composite was reported to exert a good antimicrobial activity against *S. aureus* as well as *E. coli*, and the authors demonstrate the synergic action of all included components in the structure as being responsible for improved effect [49]. Another biofilm-destructive solution was that of polysaccharide-bound silver nanoparticles. Green synthesis of caboxy-methyl-tamarind, polysaccharide-capped silver nanoparticles was performed, and the newly designed construct has demonstrated inhibitory effects against *E. coli* and *B. subtilis* growth. The obtained effect could be efficient against bacterial biofilm formation and consolidation [50].

5. Mechanisms underlying the antimicrobial effect of natural-synthetic hybrid materials

Although consistent efforts have been made for development of hybrid, natural-synthetic designs, as well as testing their antimicrobial effects, there is still limited data regarding the

exact mechanisms involved in the obtained antimicrobial effects. However, the natural compound in the construct can be considered as an important contributor in the final bacterial inhibition mechanism. The most important antimicrobial mechanisms involved in natural extract action, along with studies detailing the effect, are summarized below.

5.1. Membrane permeabilization, membrane potential alterations, and cellular component leakage

One of the most incriminated antimicrobial mechanisms used by natural extracts involves the functional and structural integrity of the membrane. Alteration of bacterial membrane potential demonstrated by Saritha et al. is a study focused on different extracts. *Leucas aspera, Hemidesmus indicus, and Plumbago zeylanica* ethanolic extracts revealed different mechanisms of membrane functional attack. While data on the ethanolic extracts of *Hemidesmus indicus* and *Plumbago zeylanica* revealed disruption of membrane continuity with leakage of cellular content and consecutive alteration of membrane potential, extract of *Leucas aspera* demonstrated functional alteration properties, with limited anatomical destruction consecutive to exposure. The latter was found to generate inner membrane alterations with preservation of outer membrane continuity, therefore lacking complete permeabilization. The effects were studied on *E. coli*. Authors provided evidence on green emission fluorochrome leakage as well as electron microscopy evidences of membrane blebbing with release of cellular contents. Such events could be possibly explained by the presence of flavonoid and phenol antioxidant molecules in the extracts, known to exert a detergent-like effect. Moreover, the antimicrobial effects were proven to be dependent on concentration and time [51]. Similarly, morphological changes such as cell membrane tearing with interference with the cell's survival were found by researchers as a mechanism used by *Polygonum cuspidatum*, a Chinese age-old therapeutic plant. The extract induced significant morphological changes such as: membrane rupturing, and content release into the exterior all followed by cell death, as images provided by scanning electron microscope have revealed [52].

Detailed evidences of protein leakage were brought by a distinct research group, while testing the effects of *Cocos nucifera* extract. The effects after bacterial exposure were analyzed from the minimal inhibitory concentration, protein potassium ions leakage from cells as well as nucleotide release following membrane permeabilization. Results demonstrate significant antimicrobial effects. Calculated MIC was ranging between 0.39 and 12.5 mg/mL. The time-kill analyze identifies 15 min as the minimal time interval for bacterial death following exposure, with 27.8% rate of death. For a concentration of 1 × MIC, protein leakage at the identified time point was ranging between 3.56 and 19.08 μg/mL, potassium ions leaked between 0.182 and 0.379 mg/mL while the nucleotides ranged between 0.609 and 2.446 μg/mL [53]. In a similar manner, the assessment of the effects exerted by *Veronica montana* L. extract was performed by researchers. Various bacterial strains, including Gram-positives and Gram-negatives were tested, by monitoring their sensitivity to exposure to extract. The most sensitive strain has been *L. monocytogenes,* and the mechanism suggested by testing data was direct lysis of pathogenic cytoplasmic membrane [54]. Also, another research group has studied the effects of monocaprylate, as a naturally generated molecule. The team focused on the mechanisms underlying

the death induced by monocaprylate on different strains, such as *E. coli*, *Staphylococcus xylosus*, and *Zygosaccharomyces bailii*. Cell morphology and content, as well as continuity of membrane were examined. Different methods, such as atomic force microscopy and propidium iodide staining, were used to depict the mechanisms. Also, by means of quartz crystal microbalance measurements, the authors have measured the concentration of monocaprylate in the samples. Based on obtained data and theoretical considerations, the authors have reasoned that the sensitivity of the membrane itself plays a role in the molecule-membrane interaction. Lipidic componence, fluidity of membrane as well as the sphericity of the membrane may play an important role. It has been demonstrated that the destructuring of membrane by the chosen testing molecule is done by increasing the amount of membrane and the fluidity level [55]. Moreover, in a recent study, intensive oxygen reactive species generation, with consecutive membrane destabling and protein leakage, was found following exposure of *Salmonella typhimurium* (as well as other strains) to the methanolic extract of *Scutellaria barbata* (*S. barbata*). The mechanisms resulted in a 24.7% death rate in the exposed bacteria following 40 min of treatment The results add a new physiopathological element to the mechanistic chain responsible for antimicrobial effects, by demonstrating the involvement of oxidative stress in the early onset of membrane alterations and content release responsible for bacterial death [56].

5.2. Alterations in regulation of gene expression

The release of bacterial cell content as a result to treatment-induced permeabilization is preceded by enhanced expression of different proteins. Yong et al. have identified several distinct proteins with up-regulated expression following medicinal plant exposure, namely chaperonin (60 kDa), flagellin, triacylglycerol lipase, outer membrane protein A, N-acetylmuramoyl-L-alanine amidase, 30S ribosomal protein s1, and stringent starvation protein A. The paper suggests common antibacterial routes for different natural antimicrobial treatments [57].

Similarly, evidences provided by El-Hamid et al. support the conclusion of inducing down-regulation of quorum-sensing system. Altering bacterial communication, exerted by plant natural therapies was demonstrated by qRT-PCR and was reported to be induced by down-regulation of quorum-sensing already established genes [58]. Also, transcription processes as well as replication of nucleic acids (DNA/RNA) were reported [59].

5.3. Metabolic alterations

Besides the already discussed mechanisms, a recent paper has discussed the addition of metabolic-induced alterations by exposure to natural extracts. The mechanisms identified by the authors were respiratory enzymatic inhibition, inducement of oxidative stress, heat-shock state, and forcement of bacterial acute stringent response. The ATP level tends to decrease in the cell, as demonstrated by the *E. coli* strain O_{157}:H_7 used by the authors, following exposure to different natural-source extracts such as: thymol, carvacrol, (þ)-carvone, or trans-cinnamaldehyde [59].

5.4. Effects induced by nanoparticles

The antimicrobial effect of particular nanomaterials represents a complex interaction of distinct effects. Modulation of effects could theoretically come from the cell internalization of free

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ions resulting from the nanomaterials, cell-nanostructure interaction, and physical properties of the nanostructures such as dimension, morphology, or surface charge. Due to the large area provided by the surface of nanoparticle, the different chemical nature of nanostructures and the final effects are hard to predict and therefore represent a serious research aim for each individual type of nanomaterial [60]. Among antibacterial applications, silver nanoparticles represent a major fraction of tested materials due to widely accepted and traditionally known effects of silver. For this nanomaterial, in particular, effects are mainly due to silver ion uptake, resulting in DNA toxicity and membrane damage [60].

6. Drawbacks, limitations, and future research trends

The mounting of medicinal resistance in bacteria and the constant changes in bacterial mechanisms against antibiotics trigger the need for different solutions, which would include a natural-based antimicrobial component. Present limitations, however, come from the little interest of pharmaceutical companies in integrating nature-provided elements into their fabrication process. Extraction and testing by specialized companies could provide an additional solution for antibacterial treatment, and should be focused on age-old validated plants used in traditional medicine [61].

The future of research within the discussed topic is dependent on improved mechanistic understanding at the interface between material and bacterial cell, as well as more in depth knowledge on nanomaterials and their specific behavior in different conditions. The more knowledge acquired, the more complex and tailored the structure of the future constructs will be. Concepts of future structures are becoming themselves a research topic necessary for generation of better nano-antimicrobial constructs [62].

7. Conclusions

The advances in nano-antimicrobials based on synthetic-natural (hybrid) designs join the achievements of two domains already demonstrating promising data for future biocide agents. Up-to-date literature suggests acceleration along the path of generating new antibacterial agents, capable to respond to the problem of severe resistance to conventional antibiotics and holding good promises for the future of the domain. Most concepts of synthesis protocols demonstrate practical efficiency, comparable with the standard recommended antibiotic treatment. However, while most polymer-based and liposomal designs were meant for textile and packaging treatment, functionalization of nanoparticles with naturally active compounds seems to suit direct antimicrobial treatment better, including the possibility for adding topics in human-intend applications. Distinctly, capsular products benefit from digestive transit protection of active components, thus making them perfect for oral administration. Concept design results in specific tailoring of final product; therefore, the choice of technology and prototype remains to be made based on the final desired application.

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