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Review Article

SILVER NANOPARTICLE INCORPORATED GEL FOR TOPICAL BACTERIAL TREATMENT

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ABSTRACT

Numerous pathogenic bacteria are developing antibiotic resistance. Improvement from multidrug resistant (MDR) infections is a problematic issue and requires a multiple treatment with broad-spectrum antibiotics which are less efficient, more toxic and more expensive. Silver nanoparticles (AgNPs) are attracting much attention because of their potent antibacterial and anti-biofilm activities. These nanoparticles are lengthily synthesized and used as a successful broad spectrum antibacterial agent against Gram negative and Gram positive bacteria including antibiotic unwilling bacteria. Silver attacks multiple targets in the microorganism consequently decreases its ability to develop battle. Effect of coating on AgNPs constancy, toxicity on the microbial host and their antimicrobial activity was discussed. The amalgamation of AgNPs into hydrogels magnifies the antibacterial activity according to their characteristics; hydrogels work as an competent stabilizer of AgNPs and control the release of AgNPs. In the present review, the silver nanoparticles-hydrogel and their components as well as their antibacterial activities and wound healing efficacies are reviewed and discussed on the bases of constituent variabilities and characteristics. Different factors and topics are measured since there are challenges and challenging issues need to be faced and solved for expansion of new ideal antibacterial formulation.

Keywords: Bacterial resistance, Silver nanoparticles, Antibacterial agents, Wound healing, Hydrogel, Coating agents.

1. INTRODUCTION

Nanostructured particles (NPs) and the three dimensional hydrogels have been extensively involved the attentions of many authors and investigators as promising materials in the biomedical fields owing to their unique structures and properties as well as the growing needs of new efficient non-traditional antimicrobials [1-4]. Metallic nanoparticles including gold, silver, platinum, cobalt, nickel and copper and metal oxide NPs including iron oxide (Fe_3O_4, Fe_2O_3), titania (TiO_2), zinc oxide (ZnO), cupric oxide (CuO) as well as metal alloys and salts are of great attention. The scientific research community has dedicated widespread efforts to develop appropriate synthetic techniques for producing nano-particles taking in consideration synthesis restriction by the environmental pollution caused by heavy metals [5]. Nanoparticles-loaded hydrogels can be made throughout the amalgamation of NPs and hydrogels via various mechanisms which were discussed by Thoniyot, et al. [3]. Besides, numerous studies and investigations were carried out in concern with the different components, types and characteristics of hydrogels as well as the physicochemical characteristics and methods of synthesis of nanoparticles [6-9]. Furthermore, these variable studies postulated and discussed the efficacies of the nanoparticle hydrogel as antimicrobial agents against bacteria, virus, and fungi particularly those with resistance to antibiotics. The submission and use of nanoparticles as antibacterial agents were the focus of many authors [10, 11]. Recent advances in the design, synthesis, functionalization and function of nano-composite hydrogels with enhanced mechanical, biological and physicochemical properties were reviewed by Zhao, et al. [4]. In admiration with nano-hydrogel related entity components, dissimilar other reviews are reported [1]. These reviews referred to some drawbacks in the current studies such as the absence of *in vivo* application on animal models and of their rheological distinctiveness. The optimization of the constituents of the NPs hydrogel composites is essential on the light of their target as antibacterials and biofilms in medical use especially in wound healing since the skin and its characteristics symbolize an imperative factor. Among the metallic nanoparticles, AgNPs are attracting much interest because of their potent antibacterial and antibiofilm activities [1]. These nanoparticles are

comprehensively synthesized and used as an efficient broad spectrum antibacterial agent against gram negative and gram positive bacteria including antibiotic resistant bacteria [12]. Coating of AgNPs has been applied by many authors using different polymers mainly for their stability and for different other purposes including reducing nanoparticle toxicity on the microbial host and obtaining benefits of coats as antimicrobials [13, 14]. The production of hydrogels and the incorporation of AgNPs into these hydrogels were investigated by several authors [1, 4, 8, 15-17]. Accordingly, the choice of gelling agents especially with antibacterial activity and their use in hydrogels are of great interest to augment the antibacterial effectiveness of AgNPs in synergetic and optimized exploit toward producing an ideal hydrogel [18]. According to the aforementioned conclusion and reviewing literature, there are diverse factors and topics to be studied in relation to the production of ideal AgNPs hydrogel composites mainly for topical application.

2. BACTERIAL ANTIBIOTICS CONFRONTATION

Several pathogenic bacteria are increasing antibiotic resistance [19-22]. Data on the resistance patterns for the bacteria of public health significance were reported by World health organization in all regions with nationwide data of *Escherichia coli (E. coli)*, *Klebsiella pneumonia* and *Staphylococcus aureus*, the quantity of resistant to the normally used antibacterial drugs exceeded 50% in many settings. The confrontation of the first two species in some European Union (EU) countries is already high and increasing whereas the latter species in the past few years has shown either a unremitting decrease or stabilizing trend in most EU countries with a percent above 25 in more than one fourth of the reporting country. Recovery from multidrug resistant (MDR) infections is a problematic issue [23, 24] and requires a multiple behavior with broadspectrum antibiotics which are less effective, more toxic and more luxurious [24]. Different authors reviewed, discussed and made trials to find solutions to bacterial confrontation to antibiotics [23-27] throughout the history of infectious disease which can be divided into 3 eras: the pre antibiotic era, the antibiotic era, and the era of promising infectious diseases [28]. During these era, antimicrobial confrontation was recorded, so it is not new, but the number of resistant organisms, the geographic locations affected by drug confrontation, and the wideness of resistance in single organisms are unprecedented and mounting [29]. Levy, Marshall [26] stated that clinically significant bacteria are characterized not only by single drug resistance but also by multiple antibiotic resistances. Increasing rates of bacterial resistance among common pathogens are threatening the effectiveness of even the most potent antibiotics and created a major public health dilemma, compounded by a dearth of new antibiotic options [27]. The latter author reported that multidrug-resistant Gram-negative organisms have received less attention than Gram-positive threats, such as methicillin resistant *Staphylococcus aureus*, but are just as menacing. The increasing mortality and morbidity rates of Methicillin resistance *Staphylococcus aureus* (MRSA) infections and its being the main cause of worldwide nosocomial infections gave it a great medical importance [30]. In general, *Staphylococcus aureus* causes superficial lesions in human skin, localized abscesses, septicemia, pneumonia and bacteremia producing between 25-63% of mortality [31]. Skin cuts, abrasions and burns are susceptible to MRSA infections that might dramatically lead to significant morbidity or life threatening conditions [32]. Moreover, bacterial infection plays a negative effect on dermal wounds and hence it is essential to control the wound free of bacteria [33]. The biochemical and genetic aspects of antibiotic resistance mechanisms in bacteria were reviewed by Dzidic, et al. [25]. Numerous genetic loci associated with antibiotic resistance were identified in a wide variety of bacterial pathogens [34].

3. ANTIBIOTICS MECHANISM ON BACTERIA

Common antibiotics generally work on different targets in the bacteria including bacterial cell wall, cell membrane integrity, DNA synthesis and integrity, RNA and protein synthesis. The haphazard use of antibiotics promoted the progressive increase of the bacterial resistance which was defined by the food technology institute of England as the ability of microorganism to remain alive and grow under destructive conditions [35]. As long as bacteria was not killed by the used antibiotic, bacteria acquire resistance by various ways including modifying the antibiotic target by genetic mutation, enzymatic destruction of the antibiotic before it gets into the cell, enzymatic modification and inactivation of the antibiotic pump the antibiotic out of the cell by specific and nonspecific transport proteins. Once resistance is developed, bacteria share and transfer it either vertically (parent-to-offspring gene transfer) or horizontally between species (cell-to-cell transfer of small pieces of the genetic information) via either transduction, conjugation or transformation [36]. The authors discussed several strategies in an attempt to overcome

bacterial drug resistance including either using compounds in combination with antibiotic that inactivate the bacterial resistance enzymes or alter the structure of the antibiotic to sterically hinder the action of modifying enzymes. The introduction of new antibiotics has not kept pace with the increasing rate of resistance, leaving clinicians with fewer treatment options. In the 1990s, when Gram positive pathogens were largely responsible for antimicrobial resistance, antimicrobial agents such as linezolid (Zyvox) and quinupristin/dalfopristin (Synercid) were developed to treat them [27]. Few new effective antibiotics were developed and approved for Gram-negative infections [37]. Lengthy and/or inappropriate antimicrobial therapy allows microbes to mutate into new forms that help them survive antibiotics and quickly become new, dominant strain. Accordingly, while mutations and production of new antibiotic resistant strains and search for new antibiotic options continues, there is urgent need to employ different strategies that will slow the development of resistance to the current armamentarium, such as avoiding prolonged antibiotic use or under dosing, using pharmacokinetic and pharmacodynamic principles to choose dosing regimens, and encouraging early and aggressive empirical therapy. These action should be followed by de-escalation and narrowing the antimicrobial spectrum when culture results become available [27]. Unfortunately, resistance is not the only challenge for antibiotics to develop their actions. Bacteria protect their colony by forming a biofilm which is self-produced exocellular matrix layer. Once this biofilm is formed, antibiotic dose must be elevated 1000 time to overcome the bacterial colony [23]. So, there are continuous challenges in this aspect to solve such elevated problematic issues of antibiotic resistance depending on further understanding of their mechanism and finding of new strategies of treatments.

4. ANTIMICROBIAL BATTLE IN BIOFILM

Bacteria traditionally were manipulated and treated as free-floating planktonic replicating cells. Recently, it is recorded that about 99% of all bacteria on the earth are living in spatially distinct and organized communities, referred to as biofilms, a form in which they behave very differently with only 1% living in the planktonic state [38]. An estimation of 65% of microbial infections was found to be associated with biofilms representing one of the hottest topics in microbiology [39]. Moreover, the National Institutes of Health (NIH) has estimated that up to 80% of human infectious diseases are biofilm related [40]. The biofilm bacteria are contained in a self-produced polymeric matrix made principally of exopolysaccharide. This matrix contains polysaccharides, proteins and nucleic acids originating from microbes often making up to 80% of the biofilm [41]. The bacterial consortium can consist of one or more species living in a sociomicrobiological way [38]. Biofilms can be composed of a population developed from a single species, or from a community derived of multiple microbial species. Microbial communities natively populate human mucous membranes and epithelial surfaces such as the gastrointestinal tract, oral cavity, and skin. Some general features of biofilm infections in humans compared with acute planktonic infections are given by Høiby, et al. [42] with emphasize on their tolerance to immune response and clinically dosing of antibiotics. The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of antibiotics to biofilm-growing bacteria being up to 100-1000 fold higher than for planktonic bacteria [38]. Despite the longtime of research, very little is known about the molecular mechanisms of antibiotic resistance in biofilms. However, recent studies have used a variety of model systems to determine how and why biofilms are so resistant to antimicrobial agents; a question to be answered [43]. Although several theories have been proposed, the precise mechanism of how this sensitivity is altered has still not been clarified [38]. Nevertheless, it is possible to separate these mechanisms into intrinsic (or innate) and extrinsic (or induced) resistance factors. These factors are reviewed by many authors including Paraje [38] and Mah,O' Toole [43] with the conclusion that bacteria develop successful strategies for survival which include attachment to surface and development of protective biofilms. These successful strategies make it difficult to control biofilm growth, with a biofilm providing bacteria with a 10- to 1,000-fold increase in antibiotic resistance compared to free ones [38]. New strategies are required to overcome extreme biofilm antibiotic resistance by the development of novel therapies aimed at disrupting biofilms especially in their deeper layers [44] and killing the constituent bacteria, with manipulation of intrinsic and extrinsic resistance pathways providing much promise for future treatment of biofilm infections in spite of biofilm defenses against ultraviolet light, biocides, antibiotics and host defenses.

5. BACTERIAL METAL ION CONFRONTATION

Metals are naturally available due to the natural geographical events and the volcanic activity. Therefore, bacteria are exposed to both essential and toxic

bioavailable metals. This exposure has been the promotor for the increased ability of microorganisms to decrease the cellular level of those naturally bioavailable oxidized metal ions. Metals such as mercury, arsenic, copper and silver have a long empirical history of human usage in medicine especially as antimicrobials for thousands of years, despite problems of host toxicity or doubts about their efficacy [45]. However, except for silver use in burn treatment, there were general decline in the clinical use of antimicrobial metal compounds after the discovery of antibiotics and new other antimicrobial compounds. The antimicrobial resistance of a wide range of metals was reviewed and briefly discussed by Hobman, Crossman [45] especially in concern with mechanism of metal ion toxicity on bacteria [46, 47], bacterial homeostasis [48], mechanism of antimicrobial metal resistance [49-51] and the bacterial mobile genetic elements [52]. The silver is reported to be highly toxic to bacteria and to be the second toxic metal to *E. coli* [53]. It was firstly used as an antibacterial agent 2000 years ago in drinking water containers [51]. The bacterial silver resistance was reviewed by Clement, Jarrett [54], Chopra [49] and Silver, et al. [51]. Chopra [49] reported that gene mutation and plasmid acquisition are the most likely mechanisms of bacterial silver ion resistance in an attempt to control silver on uptake and increase its efflux. They also stated that clinician must choose a dressing that could release high concentrations of silver ion to obtain rapid antibacterial activity.

6. SILVER NANOPARTICLES

Researchers are directed towards discovering new strategies and antimicrobial agents to solve the problematic issues related to bacterial resistance to the commercial antibiotic [55]. The idea of using nanotechnology in medicine was introduced as new strategy in this concern due to the novel size-related physicochemical properties of the nanomaterials produced at nanoscale (1- 100 nm) [56]. The application and use of nanoparticles as antibacterial agents attracted the attention of many authors [10, 11]. Franci, et al. [1] reviewed the potential use of antibacterial silver nanoparticles as summarizing the emerging efforts to address current challenges in the treatment of infectious diseases and their solutions. The natural antibacterial properties of silver are strengthened at the nanoscale, so the most nanoparticles produced are made from Ag or combination of Ag and other compounds [11, 57, 58]. Silver nanoparticles have emerged as antibacterial agents owing to their function as depot for Ag ions, their high surface area to volume ratio and the unique chemical and physical properties [33, 55]. AgNPs are the most widely used antibacterial nanoparticles in various field. In comparison with silver ions in the form of AgNO3, silver nanoparticles were proved to be significantly more effective as antimicrobial agent [10]. These silver nanoparticles exhibited higher toxicity to microorganisms versus their lower toxicity to mammalian cells when compared to other metals [59]. Silver nanoparticles are produced extensively and used as effective broad spectrum antibacterial agent against Gram negative and Gram positive bacteria including antibiotic resistant bacteria [12]. Chernousova, Epple [60] stated generally that the effect of silver towards the bacterial cells was overestimated while their effect towards human cells is underestimated. However, different studies were reported to investigate the toxicity of silver ions and silver nanoparticles towards human cell lines. AgNPs are reported to be less toxic than silver ions [61]. This might be due to the limited release rate of silver ions from the surface of the nanoparticles which resulted in higher lethal dose to different cell lines than that of Ag+ (below 5μg/mL) [62]. Additionally, Lu, et al. [63] reported that 100μg/mL colloidal silver nanoparticles were not toxic to the human skin HaCaT keratinocytes (a transformed human epidermal cell line) as they showed normal cell viability after treatment with AgNPs for 24 hours. However, silver nitrate (which is the starting material for synthesis of AgNPs) showed high toxicity even at low concentrations upto 10μg/mL.

6.1. Antibacterial mechanism of silver nanoparticles

Silver attacks multiple targets in the microorganism therefore decreases its ability to develop resistance [64-66]. It was thought that bacteria can not generate resistance to silver, thus it was an important advantage for an antibacterial agent [67]. Although MRSA exhibits several resistant mechanisms that are not present in the non-MRSA, silver nanoparticles are not affected by their drug resistance mechanisms and inhibit the growth of both MRSA and non-resistant *staphylococcus aureus* in a bactericidal manner rather than bacteriostatic (MBC/MIC ratio ≤ 4) [31]. Markowska, et al. [61] reported that the antimicrobial mechanisms of ionic silver and silver nanoparticles are different. However, AgNPs are considered to have the same antibacterial mode of action as Ag ions since, they act on the same molecules and structures in the bacterial cell [67]. The antibacterial activity of AgNPs was due to the oxidative damage which

is the first toxicity developed in the form of reactive oxygen species [55, 68, 69]. AgNPs attach to the bacterial cell wall, penetrate it, cause structural changes in the cell membrane and uncontrolled increase in its permeability, leaking of the cellular content and leading to cell death [11, 31, 70, 71]. Besides, they form free radicals that damage cell membrane and cell death. Silver ion represents a soft acid that naturally tends to interact with the soft bases in the bacterial cell such as sulfur and phosphorus thus lead to cell death. Also, AgNPs act on sulfur and phosphorous, major components of DNA, leading to DNA destruction and prevention of its replication and cell division. Nanoparticles dephosphorylate the phosphotyrosine of bacterial substrate leading to inhibition of the signal transduction and stop the bacterial growth. Rawashdeh, Haik [67] reviewed the antibacterial mechanisms of metallic nanoparticles with emphasis on AgNPs and their shapes and sizes. Under aerobic conditions intracellular Ag+ ions also cause reactive oxygen species generation and interference with DNA replication increased membrane permeability and increased sensitivity to antibiotics. Du, et al. [71] reported that the membrane damage of *E. coli* caused by AgNPs was more significant under aerobic condition than under anaerobic condition. Moreover, respiratory tract inhibition is caused by silver ions, followed by membrane damage and proton motive force destruction [45]. The major toxicity mechanism is due to interaction of silver ions with the thiol groups present in membrane proteins and enzymes, especially the respiratory chain enzymes [47]. Both, ionic and nanoparticle silver were found to disrupt the outer membrane leading to collapse of the cytoplasmic membrane potential, followed by levels depletion of the intracellular ATP in *E. coli* and respiratory chain interference [10,71]. Generally, AgNPs interact with a wide range of molecular processes within microorganisms resulting in a range of effects from inhibition of growth, loss of infectivity to cell death [71] which depends on shape [64], size [56, 70], concentration of AgNPs [57] and the sensitivity of the microbial species to silver. The smaller size within the range of 1-100 nm and the truncated triangular shape of AgNPs exhibited the better antibacterial and anti-biofilm efficacy. Higher concentration of AgNPs is required to inhibit the growth of the Gram positive bacteria such as *Vibrio cholera* compared to the concentration to inhibit Gram negative bacteria like *E. coli*, the highest concentration above 75μg/ml completely inhibited the growth of all types of bacteria [57]. AgNPs-induced morphological variations in some bacteria were evident reflecting the variability of their antibacterial mechanisms [67]. Du, et al. [71] investigated the effect of AgNPs on *E. coli* cells using Transmission Electron Microscopy (TEM) and Field Emission Scanning Electron Microscope (FESEM) and showed severe cell membrane damage represented by the collapsed cell structure. Shrinkage and separation of bacterial cytoplasmic membrane was observed under TEM after treatment of *E. coli* and *S. aureus* with 0.2% Ag ions for 2 h, this led to release of the cellular content and degradation of cell wall. The anti-biofilm activity of AgNPs has been demonstrated in a number of studies [1]. Markowska, et al. [61] referred to and discussed the AgNPs-induced significant decrease in the biofilm biomass of certain bacteria in relation to nanoparticle size. Moreover, the ability of AgNPs to block bacterial growth and glycocalyx formation was proved by using confocal laser scanning microscopy (CLSM) techniques to analyze the biofilm formed by the MRSA, *S. aureus* and methicillin resistant *Staphylococcus epidermidis* isolated from infected wounds [1].

6.2. Silver nanoparticles-antibiotics connections

Different authors studied the effect of addition of silver nanoparticles to the antibiotics as antibacterial therapy. Silver nanoparticles were found to enhance the antibacterial and antibio film activities of conventional antibiotics [61, 71. The AgNPs-antibiotics action was either synergistic (Ceftazidime, Penicillin G, Amoxicillin, Erythromycin and Clindamycin), additive (kanamycin) or antagonistic (chloramphenicol) antibacterial activity. Gurunathan, et al. [21] reported that AgNPs have a significant antibacterial activity that could be used to enhance the action of antibiotics against Gram positive and Gram negative bacteria. Birla et al. [19] also, reported a strong antibacterial activity of antibiotics upon addition of AgNPs in the case of antibiotic resistant bacteria with conclusion that nanoparticles are the best solution for the increased bacterial resistance. Moreover, lower doses of the antibiotics will be needed in treating patient when combined with silver nanoparticles.

6.3. Hydrogel

Hydrogels recently attract the attention of many authors and investigations [4, 8, 15, 16]. Hydrogels, the three-dimensional cross-linked polymer networks, are smart enough to respond the fluctuations of environmental stimuli (*p*H, temperature, ionic strength, electric field, presence of enzyme etc.) and swell or shrink accordingly, [8] without being dissolved therefore forming colloidal suspension [50]. In their swollen state, they are soft and

rubbery, resembling the living tissue exhibiting excellent biocompatibility [53]. Hence, hydrogels have widely used in different applications of pharmaceutical and biomedical engineering including tissue engineering, antibacterial factor and controlled release drug delivery system. Hydrogels can be classified into several categories depending on their source (natural or synthetic), configurations (non-crystalline, semicrystalline and crystalline), type of crosslinking (chemical or physical interaction), physical appearance (matrix, film or microsphere) and network electrical charge (neutral, ionic, amphoteric electrolyte or Zwitterionic) [18, 54, 55]. Moreover, based on the methods of preparation, hydrogels may be classified into four categories: homo-polymer, copolymer, semi-interpenetrating network and interpenetrating network [8]. Homopolymer hydrogels are cross-linked networks of one type of hydrophilic monomer unit [56], whereas copolymer hydrogels are produced by cross-linking of two co-monomer units, at least one of which must be hydrophilic to render them swellable [57]. Semi-interpenetrating hydrogels are produced if one polymer is linear and penetrates another cross linked network without any other chemical bonds between them [58]. Finally, interpenetrating polymeric hydrogels defined as intimate combination of two polymers, at least one of which is synthesized or crosslinked in the immediate presence of the other [59]. Hydrogels and their preparation methods, characterizations, pharmaceutical formulations and classifications were reviewed by different authors [4, 8, 18] with emphasis on the associated environmental conditions. These reviews also referred to the important role of hydrogels in the advanced drug delivery of the new developed and discovered therapeutic moieties. In conclusion, hydrogels have a variety of properties including absorption capacity, swelling behavior, stability and degradation, bioadhesion and bioactivity, permeability, mechanical properties optical and surface properties [11-14]. These properties make them promising materials for diverse applications [4] since theoretically most of these features can be engineered into a hydrogel system [12, 14] that works with nano-particles such as AgNPs in an optimum synergetic way as antibacterial therapy.

6.4. Hydrogels loaded through silver nano-particles

Hybrid materials can be produced by combining metal-based nanoparticles such as gold and silver with polymer hydrogels. The incorporation of AgNPs into hydrogels magnifies the antibacterial activity and alter their mechanical toughness, swelling ratio and stimuli responsiveness [4, 42]. According to their characteristics, hydrogels work as an efficient stabilizer of AgNPs and control the release of AgNPs. There is little effect on the mechanical properties of the resulting nanocomposite hydrogels by the incorporation of metal nanoparticles as long as the interactions between polymer and nanoparticles are weak [42]. Thoniyot, et al. [3] summarized, in their review, five approaches for preparation of nanohydrogel composites. These approaches are (a) hydrogel formation in a nanoparticle suspension, (b) physically embedding the nanoparticles into hydrogel matrix after gelation, (c) reactive nano-particle formation within a preformed gel, (d) cross-linking using nanoparticles to form hydrogels, and (e) gel formation using nanoparticles, polymers, and distinct gelator molecules. Thoniyot, et al. [3] postulated that nano-hydrogel mixing may produce a synergistic property enhancement of each component such as the effect of the mechanical strength of the hydrogel on decreasing aggregation of the nanoparticles. Biondi, et al. [6] in their review, focused on the most recent developments in the field of nano-hydrogel composites and the associated problematic issues. Ag-NPs bind non-specifically to bacterial membranes and other components inducing structural changes that increase membrane permeability and mitochondrial dysfunction [43]. So, the controlled release of AgNPs is necessary to sustain antimicrobial efficacy by the hydrogel composites with optimization of their characteristics for biomedical applications [3]. Such controlled-release of AgNPs provides consistent protection for a period of time, without the need to remove the wound dressings. AgNPs have been incorporated into different polymer-based hydrogels. Efforts in recent years have shifted to utilizing naturally occurring materials such as chitosan, carbo-hydrate polymers such as gum acacia and dextran, and gelatin to produce bio-compatible/degradable composite materials [3, 44-46]. In conclusion, further development and investigations of the AgNPs-loaded hydrogels are required with optimization of their components to obtain synergistic antibacterial activities between nanoparticles, gelling agents and nanoparticlescoating agents.

6.5. Silver nanoparticles and skin diffusion

The skin represents the first line of defense against a wide range of bacterial pathogens. Epidermis mainly composed of keratinocytes which are structurally organized into several layers from outside namely; *stratum corneum* (SC),

stratum licidum (SL), *stratum granulosum* (SG), *stratum spinosum* (SS) and *stratum basale* (SB). The *stratum corneum* is a unique structure of hydrophobic nature representing one of the most important barriers against external environments whereas skin derivatives on the contrary represent alternative routes of entry of the skin [47]. Skin absorption should not be confused with skin permeation, which is the diffusion of a compound into a certain skin layer nor with skin penetration which is the diffusion into deeper layers. Regarding the physicochemical properties of the nanoparticles, study of nanoparticles penetration through the skin is of great concern due to their small size. Poland, et al. [47] reported in their review that nanoparticles with size more than 100 nm do not penetrate through *stratum corneum*. Particle size is not the only factor that influences the level of dermal penetration. Surface chemistry may also play a role in the penetration of nanoparticles to skin layers. Since, skin is amphoteric in nature and has an isoelectric point which is typically between 3 and 4. At physiological *p*H (*p*H 7.4), skin is considered a negatively charged surface [48] and hence it selectively permeates cations [149]. Ryman-Rasmussen, et al. [50] studied the effect of surface charge on the penetration of quantum dots (QD 565) through intact porcine skin after topical application of neutral PEG coated QD 565 (pH 8.3), anionic COOH-coated QD 565 (pH 9.0) and cationic PEG-amine coated QD 565 (pH 8.3) with pHs of the solutions that are above the isoelectric point of the skin. Using confocal microscopy, they found that PEG- and PEG-amine coated QD 565 penetrated the SC and localized within the epidermal layers after 6 h while there was no evidence of penetration of COOH-coated QD 565 after 24h. However, different authors reported that negatively charged nanoparticles penetrate skin greater than the neutral and the positively charged nanoparticles [51] that conflict with the previously mentioned cation selectivity of the skin. So, it could be concluded that the interaction of skin with charged nanoparticles is affected with other factors such as the nature and the *p*H of the vehicle and particles stability and aggregation [47]. The level of hydration of the skin, the integrity of all its layers, the presence of cuts or any skin disease is likely to influence the level of absorption of a compound through the skin. It is importantly to consider the species in the permeation study where Magnusson, et al. [52] found that the skin permeability in pig and rat were up to 4 and 9 times respectively when compared to human. So, using the rodent as animal model presents higher permeability over that in human. TEM was used to apply a qualitative microscopic visualization to confirm the presence of nanoparticles within the skin epidermal tissue and enable the determination of penetration level of particles [53]. The evaluation of penetration depth of particulate into the skin using microscopy is required to determine the systemic availability of compounds and its risk.

7. CONCLUSION

On the bases of the aforementioned review and discussion, one can conclude that the continuous development of new antimicrobial drugs and therapies is a must in parallel with the advances in biotechnology and microbiology. To have an ideal topical AgNPs-loaded hydrogel, researchers should manipulate the characteristics of their components such the nano-particles, coating and gelling agents especially those having antibacterial activities and working in synergetic mode with AgNPs. The application of different formulations of these composites on animal models is important for evaluation and assessment of therapy *in vivo* taking consideration of the human nature.

Conflict of interest

None declared

8. REFERENCES

1. Franci G, Falanga A, Galdiero S, Palomba L, Rai M, Morelli G, Galdiero M. *Molecules,* 2015; **20:**8856-8874.
2. Singh A, Sharma PK, Garg VK, Garg G. *International Journal of Pharmaceutical Sciences Review and Research*, 2010; 4:45-60.
3. Thoniyot P, Tan MJ, Abdul Karim A, Young DJ, Loh XJ. *Advanced Science,* 2015; **2:**34-50.
4. Zhao F, Yao D, Guo R, Deng L, Dong A, Zhang J. *Nanomaterials*, 2015; **5:**21-50.
5. Singh D, Rawat D, Isha. *Bioresour Bioprocess,* 2016; **3:**71-90.
6. Biondi M, Borzacchiello A, Mayol L, Ambrosio L. *Gels,* 2015; **1:** 12-30.
7. Challa R, Ahuja A, Ali J, Khar R. *AAPS Pharm Sci Tech.,* 2005; **6:** 329- 357.
8. Das NA. *International Journal of Pharmacy and Pharmaceutical Sciences,* 2013; **5:** 41-50.
9. Ghavami Nejad A, Park CH, Kim CS. *Biomacromolecules,* 2016; **17:**1213-1223.
10. Lok CN, Ho CM, Chen R, He QY, Yu WY, Sun H, et al. *J Proteome Res.,* 2006;**5:**916-924.
11. Sondi I, Salopek-Sondi B. *J Colloid Interface Sci.,* 2004; **275:**177-182.

12. Percival SL, Bowler PG, Dolman J. *Int Wound J.,* 2007; **4:**186-191.
13. Jurašin DD, Ćurlin M, Capjak I, Crnković T, Lovrić M, Babič M, Horák D, Vinković VI, Gajović S. *Beilstein J Nanotechnol.,* 2016; **15:**56-80.
14. Sharma VK, Yngard RA, Lin Y. *Adv Colloid Interface Sci.,* 2009; **145:**83-96.
15. Kirchmajer DM, III RG, In Het Panhuis M. *J Mater Chem B.,* 2015; **3:**21-40.
16. Ullah F, Othman MBH, Javed F, Ahmad Z, Akil HM. *Materials Science and Engineering: C,* 2015; **57:**414-433.
17. Yap LS, Yang MC. *Biointerfaces,* 2016; **146:**204-211.
18. Ahmed EM. *J Adv Res.,* 2015; **6:**105-121.
19. Birla SS, Tiwari VV, Gade AK, Ingle AP, Yadav AP, Rai MK. *Lett Appl Microbiol.,* 2009; **48:**173-179.
20. Brandt O, Mildner M, Egger AE, Groessl M, Rix U, Posch M, et al. *Nanomed Nanotech Biol Med.,* 2012; **8:**478-488.
21. Gurunathan S, Han JW, Kwon DN, Kim JH. *Nanoscale Res Lett.,* 2014; **9:**373-389.
22. Keat CL, Aziz A, Eid AM, Elmarzug NA. *Bioresour Bioprocess.,* 2015; **2:**1-11.
23. Vasilev K, Cook J, Griesser HJ. *Expert Rev Med Devices,* 2009; **6:**553-567.
24. Webb GF, D' Agata EMC, Magal P, Ruan S. *Proc Natl Acad Sci U S A,* 2005; **102:**13343-13348.
25. Dzidic S, Suskovic J, Kos B. *Food Technol Biotechnol.,* 2008; **46:**11-21.
26. Levy SB, Marshall B. *Nat Med.,* 2004; **10:**31-42.
27. Siegel RE. *Respir Care,* 2008; **53:**471-479.
28. Peterson LR. *Clin Microbiol Infect.,* 2005; **11:**4-16.
29. Levy SB. The antibiotic paradox: How the misuse of antibiotics destroys their curative powers (2nd edn). Cambridge: Perseus Publishing; 2002.
30. Bustos-Martínez JA, Hamdan-Partida A, Gutiérrez-Cárdenas M. *Rev Biomed.,* 2007; **17:** 287-305.
31. Ayala-Nunez NV, Villegas HHL, Turrent LDCI, Padilla CR. *Nanobiotechnol.,* 2009; **5:**2-9.
32. Turbeville SD, Cowan LD, Greenfield RA. *Am J Sports Med.,* 2006; **34:**2-21.
33. Nam G, Rangasamy S, Purushothaman B, Song JM. *Nanomater Nanotechno.,* 2015; **5:**1-14.
34. Martinez JL, Baquero F. *Antimicrob Agents Chemother.,* 2000; **44:** 1771-1777.
35. Rai MK, Deshmukh SD, Ingle AP,vGade AK. *J Appl Microbiol.,* 2012; **112:**841-852.
36. Slonczewski JL, Foster JW. Microbiology: An evolving science. United States of America: W. W. Norton & Company, Inc; 2009.
37. Rice LB. *Clin Infect Dis.,* 2006; **43:**S100-S105.
38. Paraje MG. Antimicrobial resistance in biofilms. In: A Mendez-Vilas (ed.) Science against Microbial Pathogens: Communicating Current Research and Technological Advances. Spain: FORMATEX. 2011; 736-744.
39. Potera C. *Science,* 1996; **273:**1795-1797.
40. White RJ, Cutting K, Kingsley A. *Ostomy Wound Manage,* 2006; **52:**26-58.
41. Rhoads DD, Wocott RD, Percival SL. *J Wound Care,* 2008; **17:**502- 508.
42. Høiby N, Ciofu O, Johansen HK, Song ZJ, Moser C, Jensen PØ, et al. *International Journal of Oral Science,* 2011; **3:**55-56.
43. Mah T-FC, O' Toole GA. *Trends Microbiol.,* 2001; **9:**34-39.
44. Ito A, Taniuchi A, May T, Kawata K, Okabe S. *Appl Environ Microbiol.,* 2009; **75:**4093-4100.
45. Hobman JL, Crossman LC. *J Med Microbiol*., 2014; **64:**471-497.
46. Hobman JL, Yamamoto K, Oshima T. Transcriptomic responses of bacterial cells to sublethal metal ion stress. In: D H Niesand S Silver (eds.) Molecular Microbiology of Heavy Metals: Springer Verlag Microbial Monographs; 2007.
47. Holt KB, Bard AJ. *Biochemistry (Wash).,* 2005; **44:**13214-13223.
48. Courvalin P. *J Intern Med.,* 2008; **264:**4-16.
49. Chopra I. *J Antimicrob Chemother.,* 2007; **59:**587-590.
50. Silver S, Phung LT. *Annu Rev Microbiol.,* 1996; **50:**753-789.
51. Silver S, Phung LT, Silver G. *J Ind Microbiol Biotechnol*., 2006; **33:**627-634.
52. Datta N, Hughes VM. *Nature,* 1983; **306:**616-617.
53. Nies DH. *Appl Microbiol Biotechnol.,* 1999; **51:**730-750.
54. Clement J, Jarrett PS. *Metal Based Drugs.,* 1994; **1:**467-482.
55. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, et al. *Nanomed Nanotech Biol Med.,* 2007; **3:**95-101.
56. Fabrega J, Luoma SN, Tyler CR, Galloway TS, Lead JR. *Environ Int.,* 2011; **37:**517- 531.
57. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ram′ırez JT, Yacaman MJ. *Nanotechnology,* 2005; **16:**2346-2353.
58. Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D. *Nanotechnology,* 2007; **18:**1-9.
59. Zhao G, Stevens SJ. *Bio Metals,* 1998; **11:**27-32.
60. Chernousova S, Epple M. *Angew Chem.,* 2013; **52:**1636-1653.

61. Markowska K, Grudniak AM, Wolska KI. *Acta Biochim Pol.,* 2013; **60:**61-70.
62. Samberg ME, Loboa EG, Oldenburg SJ, Monteiro-Riviere NA. *Nanomedicine,* 2011; **2:**39-50.
63. Lu W, Senapati D, Wang S, Tovmachenko O, Singh AK, Yu H, Ray PC. *Chem Phys Lett*., 2010; **487:**92-96.
64. Pal S, Tak YK, Song JM. *Appl Environ Microbiol.,* 2007; **73:**1712-1720.
65. Rai M, Yadav A, Gade A. *Biotechnol Adv.,* 2009; **27:**76-83.
66. Roe D, Karandikar B, Bonn-Savage N, Gibbins B, Roullet JB. *J Antimicrob Chemother.,* 2008; **61:**869-876.
67. Rawashdeh R, Haik Y. *Dyn Biochem Process Biotechnol Mol Biol.,* 2009; **2:**25-41.
68. Hwang ET, Lee JH, Chae YJ, Kim YS, Kim BC, Sang BI, Gu MB. *Small,* 2008; **4:**746-750.
69. Khan AU. *Int J Nanomedicine,* 2012; **7:**2997-2998.
70. Kvitek L, Pana′cˇek A, Soukupova′ J, Kola′rˇ M, Vecˇerˇova′ R, Prucek R, Holecova′ M, Zborˇil R. *J Phys Chem C.,* 2008; **112:**5825-5834.
71. Rahisuddin, AL-Thabaiti SA, Khan Z, Manzoor N. *Bioprocess Biosyst Eng.,* 2015; **38:**1773-1781.