

Review

Silver-based wound dressings: current issues and future developments for treating bacterial infections

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Abstract

The rapid and continuing emergence of antimicrobial resistance is challenging our ability to effectively fight bacterial infections. This challenge is particularly felt in clinical settings where patients experience severely infected chronic wounds. Despite the increased development and reliance on silver-based dressings, the evidence underpinning their clinical efficacy is inconclusive and there remain concerns and confusion about their use.

In this review, we describe the rationale for the current use of silver and silver-based wound dressings and highlight the progress and challenges associated with developing more advanced nanoparticle-based dressings for the safe and effective delivery of silver. Specifically, we describe the development of silver nanoparticles (AgNPs) with unique characteristics that render them applicable for controlling bacterial infections while simultaneously promoting wound healing through mechanisms including the dampening of inflammation and promotion of wound re-epithelialisation. Finally, we describe the latest progress in the development of different types of hydrogels for the therapeutic delivery of AgNPs to infected wounds. The possibility of designing and establishing multimodal silver therapies for the safe and effective treatment of infected wounds would be of great benefit for wound care management and improved patient outcomes.

Introduction

Wound healing is a multi-step, highly coordinated and regulated process that occurs in overlapping phases of haemostasis, inflammation, proliferation and remodelling¹. Aimed at rapidly restoring tissue integrity and functions, these processes are highly regulated in the coordination of multiple cell types, including keratinocytes, fibroblasts, endothelial and immune cells, as well as numerous growth factors². However, open wounds are highly susceptible to bacterial colonisation which is described as the presence

of multiplying bacterial populations in close proximity with no associated clinical signs and symptoms. A heavier colonisation load, also known as critical colonisation, may predispose an individual to wound infection^{3,4}. Wound infection is described as the invasion of proliferating bacterial cells which are present at critically high levels – 10^5 colony forming units (CFU) of bacteria per gram of tissue – where the balance between virulence and host immunity tilts in favour of microorganisms^{5,6}. In this stage, infection can trigger a cascade of host responses, including presentation of active disease symptoms – tissue inflammation, pus and exudate – which requires antibacterial treatment⁷. Furthermore, excessive accumulation of bacterial cells can result in bacteria forming a collective community cluster protected by self-produced exopolysaccharides (EPS) known as bacterial biofilms⁸. Consequently, wound infection raises critical healthcare concerns as it is one of the main causative factors for delayed wound healing which can result in serious detrimental effects⁹. Our inability to effectively clear bacteria at the early stages of colonisation increases the risk of severe infection and aids the production of biofilms¹⁰.

In Australia, more than 10% of patients will acquire a chronic wound infection, adding to the significant healthcare burden of \$2.85 billion annually that is attributed to wound care^{11–13}. Additionally, biofilms are reported to be associated with 80% of chronic wounds and significantly contribute to their impaired healing¹⁴. Once established, biofilms are highly resistant and are recognised as a major contributor to the development of bacterial resistance to antibiotics¹⁵. Biofilms prevent antibiotic penetration into infected wounds and often result in treatment failure¹⁶.

In response to the increased incidence of multidrug-resistant (MDR) bacteria and the lack of new antibiotics in research and development, research focus has again shifted back to investigating the antibacterial capabilities of silver; this, however, is now with a stronger emphasis on new therapeutic modalities that can simultaneously promote tissue regeneration and control bacterial infection. The antimicrobial activity of silver has been used for hundreds of years in wound care with broad spectrum activity observed against a range of bacteria^{17,18}. However, certain limitations, including its possible adverse effects on healthy skin tissue, have restricted its widespread use and have prompted research towards optimising the therapeutic benefits of silver while minimising the potential side effects for the treatment of wounds. These developments will be discussed and summarised in this review.

The use of silver in wound healing

Silver as an antimicrobial agent

Silver is a transition metal known to have many useful biological properties and is well-recognised for its broad spectrum antibacterial effects against both gram-negative and gram-positive bacteria. Silver in its metallic state (Ag^0) is inert, while silver in its monoatomic state (Ag^+) is biologically

active and poses strong bactericidal activity¹⁹. Oxidation of silver atoms readily occurs in the presence of molecular oxygen (O_2), leading to biologically active silver ions. The medical application of silver ions has been well documented for centuries and it has been employed as the first-choice treatment for many ulcerations and infectious diseases including venereal disease, bone perianal abscess, eye drops and burn wounds^{20,21}. The discovery of antibiotics in the early 20th century led to a decline in the use of silver as an antimicrobial agent. However, the emergence of antibiotic resistance in the early 1960s led to a renewed interest in the use of silver products, including concentration-dependent (0.5% and 1%) silver nitrate (AgNO_3) for the treatment of burns, 1% topical cream formulations, for example silver sulfadiazine (Ag-SD), and many other forms of advanced silver dressings^{22,23}. Over the last 2 decades, this resurgent interest in silver has led to large-scale productions of numerous silver-containing dressings impregnated with silver salt or metallic silver, a selection of which is included in Table 1^{5,19}.

The difference in current silver wound dressings is largely based on both silver composition and the amount of active silver that is released into the wound. Mostly, the reported dressings are manufactured with the simple impregnation of silver nitrate into a dressing material that is susceptible to unregulated fast release, resulting in short-term activity and the need for frequent dressing changes²⁴. Additionally, traditional silver dressings are susceptible to rapid inactivation by wound fluid, resulting in chemical complexes that can prevent their antibacterial activity²⁵. To compensate for the potential loss of activity, the amount of silver in the wound dressings is excessively increased to replenish silver ions²⁶.

However, excessive accumulation of silver into wounds has been shown to inhibit skin cell proliferation and impair wound healing^{27,28} and *in vitro* and *in vivo* studies have shown that application of silver dressings can increase host tissue toxicity and that care must be taken for its widespread usage^{29,30}. Consequently, advances in the field of nanotechnology have enabled the possibility of developing silver in the form of nanoparticles (NPs) to deliver significantly smaller amounts of silver into the wounds, hence improving safety without compromising its excellent antibacterial properties^{31,32}. Progress in this field is reflected by the increased number of patents developing silver for medicinal applications over the last decade (2007–2017), emphasising the high potential for industrial and healthcare applications³³.

Silver nanoparticles

NPs are materials engineered with at least one dimension within the nanoscale range (1–100nm)³⁴. A one-nanometre particle represents 10^{-9} metres in length which brings a significant difference in dimension and functionality compared to bulk counterparts. The major advantage of NPs is the versatility and tunability of their physicochemical properties depending on their application to medical purposes, including

controlled drug delivery, improved efficacy and enhanced transport across biological barriers³⁵. The continuing interest in silver nanoparticles (AgNPs) is related to the bactericidal efficacy of AgNPs at a much lower concentration than silver ions, as well as decreased toxicity to mammalian cells and other unique properties enabling optimisation of surface functionality³⁶. AgNPs provide a sustained source of silver ions as the gradual oxidation of silver atoms releases biologically-active silver ions, providing a constant flux of silver that can target key bacterial pathogen structures³⁷. In contrast to conventional silver nitrate, the combined action of a continuous supply of silver ions and the optimal physicochemical properties of AgNPs pose a substantial

threat to bacterial survival when exposed to the treatment. Additionally, the versatile and dynamic properties of AgNPs provide a greater degree of flexibility in manipulating key physicochemical properties, including structure, size, surface functionalisation and biocompatibility, to achieve desirable antimicrobial benefits³⁸. In particular, the large surface area of AgNPs allows more prominent numbers of active sites for interaction with biological species and makes AgNPs a better material than usual bulk materials³⁹. The knowledge of how AgNPs and bacterial cells interact has greatly increased over the past decade and, as a result, several possible mechanisms of bactericidal action have been proposed; these are described below.

Table 1. A selection of commercial silver wound dressings

Product	Manufacturer	Dressing composition	Silver composition
Acticoat™ Flex 3 & 7	Smith & Nephew	An inner absorbent polyester layer protected between two outer layers of silver-coated polyethylene net	Nanocrystalline silver
Actisorb Plus 25	3M™ + KCI	Carbonised fabric enclosed in a sleeve of spun-bonded non-woven nylon	Metallic silver
Allevyn® AG	Smith & Nephew	Polyurethane foam bonded to upper and lower adhesive coated polyurethane film	5% silver sulfadiazine
Aquacel® Ag	ConvaTec	Hydrocolloid fibre, sodium carboxymethylcellulose (NaCMC)	1.2% (silver nitrate)
Biatain Ag	Coloplast	Polyurethane foam non-adhesive or adhesive dressing	Ionic silver
Silverlon®	Argentrum Medical	Non-adherent, knitted nylon	Metallic silver
Silvercel™	3M™ + KCI	Alginate, carboxymethylcellulose (CMC) and silver-coated nylon fibres	Ionic silver
Maxorb® Extra Ag+	Medline Industries	Calcium alginate and CMC fibres	Ionic silver
Meplix® Ag	Mölnlycke Health Care	Silver-coated foam with silicone interface	Silver sulphate
Mepitel® Ag	Mölnlycke Health Care	Silver-coated silicone	Silver sulphate
Mölnlycke Melgisorb® Ag	Mölnlycke Health Care	Alginate fibres	Ionic silver
PolyMem® Silver	Ferris Mfg. Corp	Polyurethane foam containing F-68 surfactant, glycerol and starch polymer	Nanocrystalline silver
Promogran™ Prisma Ag	3M™ + KCI	Oxidised regenerated cellulose, collagen and silver	Ionic silver
SilvaSorb® Gel	Medline Industries	Glycerol, polyacrylamide, CMC	Ionic silver
Tegaderm® Alginate Ag	3M™	Guluronic acid, calcium alginate and CMC	Silver sodium hydrogen zirconium phosphate
Urgotul® SSD	Laboratoires URGO	Polyester gauze dressing impregnated with particles dispersed in Vaseline paste	Silver sulfadiazine
V.A.C.® GranuFoam Silver	3M™ + KCI	Silver-coated polyurethane sponge	Metallic silver

Antibacterial mechanisms of AgNPs

The continuous leaching of silver ions from AgNPs is effective against a broad spectrum of bacteria, including both gram-negative and gram-positive bacteria²⁶. While it is clear that silver ions are highly reactive and possess a high affinity for organic amines, phosphates and, most notably, thiols present in DNA, proteins and cell membranes, it remains unclear whether the NPs themselves contribute to bacterial toxicity^{40,41}.

There are three currently accepted mechanisms for the antibacterial action of AgNPs. Firstly, AgNPs can physically interact with a bacterial cell membrane, causing surface corrugation and hence an increase in membrane permeability, allowing AgNPs to penetrate intracellularly⁴². Secondly, AgNPs are responsible for attacking respiratory proteins and impairing the respiratory chain, hence hindering the production of energy and causing interference with mitochondria to generate reactive oxygen species (ROS)⁴³. Finally, AgNPs can penetrate inside the bacterial cells which allows interaction of AgNPs with cellular structures and biomolecules, including lipids, proteins and DNA, leading to disruption of key cellular events and bacterial functioning⁴⁴. These important dynamic processes lead to severe impairment of essential cellular functions, ultimately leading to bacterial cell necrosis and death³⁶.

AgNPs are shown to be effective against a wide range of microorganisms, including bacteria, fungi and anaerobes, and the susceptibility differences are pathogen-specific based on the type of organism and membrane structures¹⁸. However, AgNPs mechanisms of action have largely been shown to be similar across different pathogens in targeting multiple key membrane structures to cause pathogen cell death. For example, Kim et al. report that 13.4nm AgNPs showed the minimum inhibitory concentration (MIC) against *E. coli* to be 6.6nM compared to 33nM for *S. aureus*⁴⁵. The differences depend on the peptidoglycan layer where gram-negative bacteria (i.e. *E. coli* and *P. aeruginosa*) are more susceptible than gram-positive bacteria (i.e. *S. aureus* and *S. epidermidis*). Recent studies have also proposed that the simultaneous interaction of AgNPs on multiple targets in bacterial cells makes it difficult for bacteria to develop silver resistance, a key advantage against current antibiotics^{36,46}. A more detailed review of the antibacterial mechanism of AgNPs is reported elsewhere^{19,47}.

Physicochemical properties of AgNPs enable high antibacterial activity

AgNPs exhibit particle-specific activity, where the pathways involved in the antibacterial activity are highly dependent on the physicochemical properties of NPs. The physical and chemical properties of AgNPs' structure – size and surface structure – are important for their toxicology⁴⁸. In particular, the size of the NPs is the crucial factor that influences their intrinsic antibacterial properties⁴⁹. The influence of particle size has been demonstrated in cell toxicity, tissue

distribution, dermal penetration and cellular uptake⁴¹. Morones et al. demonstrate the size dependence activity of AgNPs (1–100nm) against a range of bacteria, namely *E. coli*, *V. cholera*, *S. typhus* and *P. aeruginosa*⁵⁰. AgNPs in the range of 1–10nm showed significantly higher affinity in the attachment to the bacterial surface compared to larger sized AgNPs. Similarly, Agnihotri et al. studied the 5–100nm size of AgNPs and observed that the smallest AgNPs (5nm) showed the fastest and most efficient bacterial killing⁵¹. Additionally, many studies reveal that AgNPs smaller than 10nm provide favourable antibacterial properties^{49,52} and have a greater tendency to penetrate bacteria compared to large AgNPs⁵³. Therefore, the unique size of AgNPs is particularly relevant in response to treating bacterial biofilms.

AgNPs have also been shown to physically interact with the biofilm matrix to deteriorate and disassemble the biofilm integrity as a route to access the bacterial cells⁵⁴. The effective removal of biofilm depends on the diffusion capacity of the antimicrobial agent, where smaller NPs bring significant advantages⁴⁰. Hence, small-sized AgNPs are expected to readily diffuse through the biofilms to release a high concentration of silver ions, eradicating the bacterial biofilms⁵⁴. For example, Choi et al. show that 20nm AgNPs can penetrate up to 40µm in *E. coli* biofilm within the first hour, which is a significant advancement on the penetration of both antibiotics and silver nitrate⁵⁵. In the context of chronic wounds, AgNPs provide a significant improvement to current silver dressings which are limited to killing only planktonic cells due to the nature of silver delivery and poor penetration of bacterial biofilms associated with chronic wounds.

In addition to the size, the surface charge of AgNPs is another important factor that determines its biological effectiveness. Most microbes display an overall negative surface charge and are expected to provide a higher affinity for positively charged AgNPs due to electrostatic interaction. Recent studies have shown to enhance the retention of AgNPs on the bacterial surface via electrostatic mediated interaction^{56,57}. A recent study by El Badaway et al. demonstrates that the surface charge of AgNPs is the most important factor that governs the AgNPs' toxicity to microbes⁵⁸. Here, negatively charged, citrate stabilised AgNPs were the least toxic, whereas positively charged AgNPs (+38mV) showed the greatest pathogen toxicity. The increased adherence and accumulation of AgNPs towards the membrane causes irreversible morphological changes, resulting in the loss of membrane integrity and permeability⁵⁰. The use of surface-engineered AgNPs may also provide a superior advantage to overcome biofilm recalcitrance to antimicrobials. For example, Xiaoning Li et al. show that the neutral and anionic particles cannot accumulate on the membrane or penetrate bacterial biofilms, while cationic particles readily penetrate and efficiently dissipate bacterial biofilms⁵⁹. Collectively, it is expected that the interplay between AgNPs' size and surface structures will bring significant advancements for removing bacterial biofilms⁴⁹.

Role of silver and AgNPs in wound healing

Despite the positive anti-microbial effects of silver, negative effects on healthy tissues and cells have been observed²⁸. However, these are dependent on several factors, including the mode of delivery, silver concentration, release rate, and duration of exposure^{23,37}. A fine balance is therefore required between delivering an effective amount of silver for the elimination of bacteria that causes minimal skin damage⁵⁴.

The negative effect of AgNPs is only observed when they are used at high concentrations or for prolonged exposures²⁸. In contrast, silver nitrates, even at low concentrations, are shown to induce an excessive inflammatory response which can result in delayed wound healing⁶⁰. Similarly, clinical studies have shown that continuous application of silver sulfadiazine Ag-SD can lead to hypertrophic scars and systemic and cutaneous argyria^{61,62}. These side effects are due to the unregulated release of silver ions resulting in unnecessary tissue accumulation predisposing the host to tissue toxicity.

The widespread and prolonged use of silver dressings is still the subject of debate as the knowledge of their toxicological aspects emerges showing negative impacts on tissue integrity. The application of AgNPs could potentially overcome the current silver limitations. Tian et al. have shown that AgNPs can decrease inflammatory reactions by modulating cytokine levels and can facilitate the early phase of wound healing⁶³. Similarly, Singla et al. saw faster wound healing with improved re-epithelisation, significant expression of collagen and growth factor, and improved vasculogenesis following treatment with AgNPs⁶⁴.

Additionally, the role of AgNPs in promoting fibroblast migration and differentiation has been well documented; however, this remains contradictory⁶⁵. For example, studies have shown that AgNPs are able to promote wound healing through positive effects on fibroblast and keratinocytes migration as well as the ability to modulate cell differentiation^{66,67}. In contrast, other pre-clinical studies have shown that extracts of silver solution from commercial silver dressings induce a significant anti-proliferative effect on keratinocytes and fibroblasts⁶⁸. In fact, topical applications of silver solutions have been shown to induce adverse effects on skin cell viability which restricts its widespread use⁶⁹.

Nevertheless, silver in the form of NPs (AgNPs) offers numerous benefits beyond the antibacterial effect. In addition to its anti-inflammatory and proliferative effect, it has also been shown to down-regulate metalloproteinases (MMP) to an optimal level that facilitates faster healing⁷⁰. However, despite the significant potential offered by AgNPs, emerging clinical studies have shown conflicting evidence; a recent international consensus document on the use of silver in wound care has highlighted that future clinical studies should focus on determining the underlying mechanisms of silver ions and wound healing responses⁵.

Advanced therapeutic delivery of AgNPs

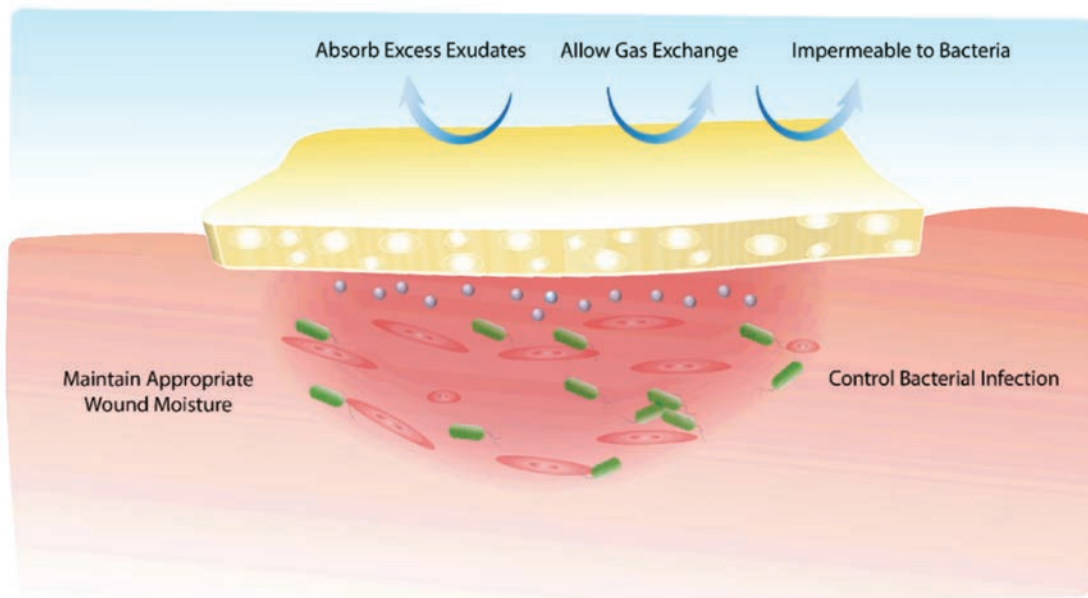
Significant effort has been invested to develop a new generation of silver-based therapies focusing on those that can eradicate pathogenic bacteria with a minimal amount of silver for an extended period. Different types of dressings, coatings, nanofibres and hydrogels have been developed to maximise the therapeutic benefits of silver⁷¹⁻⁷³. An ideal antibacterial, as shown in Figure 1, is expected to provide a moist wound environment, offer a protective role in secondary infections, remove wound exudate, and promote tissue regeneration⁷⁴.

In particular, hydrogel, a three-dimensional porous structure with high swelling capacity, has proven to be the material of choice for topical wound application capable of stimulating the body's self-healing mechanism and promoting functional tissue development by providing a suitable microenvironment for healing⁷⁵. Furthermore, hydrogels are biocompatible, have a consistency similar to the extracellular matrix (ECM), have the ability to remove excessive wound fluid, are biodegradable, and allow ease of synthesis⁷⁶. In particular, the success of hydrogel delivery systems is highlighted by improved AgNPs stability, controlled drug release profile, and enhanced localised efficacy⁵⁴. The hydrogel delivery system of AgNPs can be tailored for a prolonged delivery mode; this represents a great advantage concerning topical administration and sustained antibacterial efficacy. In addition, the physical and mechanical properties of the hydrogel are similar to the ECM, thereby presenting as an important supporting material for cellular proliferation and migration to stimulate key stages of wound healing⁷⁷. Indeed, clinical studies to date have shown that the application of hydrogel biomaterials leads to improved wound healing outcomes^{78,79}.

Several studies have shown the possibility of incorporating AgNPs in various types of the hydrogel; these hydrogels are designed to either act as a 'smart' hydrogel (pH or temperature-responsive) or a simple polymeric carrier for improved delivery of therapeutics. In particular, smart hydrogels that trigger the release of AgNPs in response to environmental stimuli are gaining significant clinical attention for their potential to provide on-demand targeted delivery⁸⁰. For example, Huang et al. have developed a multifunctional hydrogel encompassing both pH and temperature responsiveness properties while also providing extended-release of AgNPs for enhanced antibacterial effect⁸¹. Similarly, a recent *in vitro* study has shown controlled impregnation of ultrasmall AgNPs into a biocompatible thermo-responsive hydrogel for an extended-release profile with improved biofilm penetration capability⁵⁴.

Additionally, other *in vitro* and *in vivo* studies have loaded AgNPs into different kinds of carriers, some of which include chitosan hydrogel to provide a synergistic antibacterial effect⁸², polyvinyl alcohol/chitosan⁸³, and injectable hydrogel systems⁸⁴. In fact, current research is heavily focused on

Figure 1. Illustration of an ideal antimicrobial wound dressing. The polymeric multipurpose hydrogel dressing is required to not only control bacterial infection but also promote tissue regeneration



improved delivery of AgNPs using a variety of polymeric hydrogels either chemically or physically entrapped within the gel networks for slow and sustained release of silver, hence minimising the potentially toxic effects of silver². In contrast to traditional silver dressings, AgNPs loaded into biocompatible hydrogels provide significant advantages through the capability to modulate the release properties and improve antibacterial effects. The release of AgNPs in a controlled and premeditated manner can be attained by modulating the hydrogel properties including porosity, diameters and degradability. As indicated by recent research, the advancement in hydrogel technology is expected to further accelerate to exponential levels to enable new functionalities and applicability in healthcare, paving the way for the development of an ideal AgNPs-loaded antimicrobial wound care product.

Conclusion

Despite significant advances in antimicrobial wound dressings, the treatment of infected wounds remains a significant clinical challenge. Recent progress has resulted in the development of numerous types of dressings, ranging from traditional to polymeric advanced dressings for different treatment purposes. However, no dressings are currently capable of providing multifunctional properties addressing all issues in chronic wounds, including controlling bacterial infection, regulating inflammation and promoting tissue regeneration. Medical devices and wound dressings impregnated with AgNPs may constitute an advanced and superior way to control acute and chronic bacterial wound infections. AgNPs present improvements on common antimicrobial strategies to overcome the current shortfalls due to bacterial resistance and improve therapeutic efficacy compared to conventional silver products (silver nitrate, silver sulfadiazine).

However, despite the recent progress, clinical approval of AgNP-based dressings is hampered by many challenging factors including the ability to control the silver loading, maintaining release profile, specificity to biological tissue, and biocompatibility. Therefore, it is anticipated that the safety of AgNPs-based formulations will be improved when encapsulated in biocompatible carriers such as hydrogels to combine multiple characteristics that are required to facilitate wound healing and infection control. AgNPs loaded into a stimuli-responsive hydrogel are gaining a lot of attention for their improved functionalities and capabilities for topical wound applications. Therefore, AgNPs-based hydrogels are expected to have great promise in treating acute and chronic wound infections.

Conflict of interest

The authors declare no conflicts of interest.

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