Review:

A Review on Silver Nanoparticles Based Biomaterials for Bone Tissue Engineering and Medical Applications

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Abstract: *Silver nanoparticles (AgNPs) are being studied extensively as nanostructures for novel and enhanced biomedical applications. Nanoparticles in bone grafts have been shown to speed up fracture healing by providing a finer structure for bone tissue engineering. They are known to have excellent antimicrobial properties due to their high surface-to-volume ratio and small particle size, without affecting the material's mechanical properties. AgNPs' distinct property makes them the preferred filler in a wide range of biomaterials, where they play an important role in enhancing properties. AgNP-containing biomaterials can be easily tailored to meet specific requirements in the development of bone tissue. Adjusting size, shape, composition, and surface charge can enhance their effectiveness in interacting with bone cells, promoting cell attachment, and delivering growth factors. Using AgNP-based biomaterials can help prevent or reduce the formation of biofilms. The utilization of these materials in bone tissue engineering has demonstrated potential in treating musculoskeletal conditions and bone traumas. They are versatile for various applications such as bone implants, scaffolds, wound dressings, and antibiotic delivery systems. This review will cover several special topics, including silver-based biomaterials, toxic properties, morphology and mechanical properties of silver-based biomaterials, and applications of silver-based biomaterials in medical applications.*

Keywords: *antimicrobial properties; bone tissue engineering; biomaterials; nano silver particles; medical applications*

■ INTRODUCTION

Currently, tissue engineering is being employed extensively in drug development, biology research, meat production, and cell implantation for clinical therapy [1-3]. Basic tissue engineering technologies, such as cell sheet technology for creating cell tissue, have also been created by other researchers. Tissue engineering employing "cell sheets"—sheet-shaped groupings of cells with extracellular matrix (ECM)—is known as cell sheet technology. Multiple cell sheets can be layered to generate 3D dense networks of cells. Cell sheets have a sheet shape that makes them easy to handle and have high adhesive capabilities that make them easy to transplant [4-5].

Silver nanoparticles (AgNPs) possess strong antibacterial properties, capable of inhibiting bacterial growth and preventing infections within the domain of bone tissue engineering. Infections post-bone surgery can hinder the healing process, but utilizing AgNPs-based biomaterials can effectively reduce the risk of infection, leading to faster patient recovery. These biomaterials are known for their biocompatibility, meaning they do not typically trigger significant immune responses or toxicity, making them suitable for

medical applications like bone tissue engineering. They can be easily customized to meet specific needs in this field by adjusting factors such as size, shape, content, and surface charge to enhance interactions with bone cells, promote cell adhesion, and facilitate the transport of growth factors. The use of AgNPs-based biomaterials in bone tissue engineering has shown promise in addressing musculoskeletal conditions and bone injuries, and they have been applied in bone implants, scaffolds, wound dressings, and controlled antibiotic delivery systems [6-7].

Bone is a rigid tissue that supports and protects the human body's organs. It is also made up of inorganic and organic materials such as hydroxyapatite and collagen fibers. The unique interactions between these different elements produce the porosity and mechanical characteristics that define bone, which in turn give rise to the ordered structure of the skeletal system. Collagen fibers and hydroxyapatite are two examples of the inorganic and organic components found in bone, a strong tissue that supports and shields human body organs. These different components have specific connections with one another that give bone its porosity and mechanical properties, resulting in the structured structure of the skeletal system. Hence, if the bones are distorted, the integrated structure will be disrupted, which will affect human life quality [8-9].

The development of novel biomaterials that imitate bone structure is imperative due to the proliferation of degenerative disorders. This biomaterial needs to be biocompatible, non-toxic, and biodegradable in order to be implanted into the body without creating problems. Bone tissue is made up of hard connective tissue and is an important part of the musculoskeletal system. Organic and inorganic components are known to exist in bone ECM [10-11]. The organic components mainly consist of matrix proteins, proteoglycans, and various types of collagen fibers, while the inorganic components are primarily composed of hydroxyapatite. Within bone, various types of cells are present, including bone progenitor cells, osteoblasts, osteoclasts, and osteocytes [12-14].

Common injuries observed in clinical settings include bone defects resulting from trauma, tumors, infections, or bone-related disorders. Individuals with severe skeletal defects often experience disability, impacting their quality of life, work capacity, and placing a significant strain on society and the economy. These defects are categorized as small bone defects or large bone defects, with the latter being critical size defects exceeding 1.5 times the diameter of a long bone. Small non-infected bone defects typically heal independently following debridement, while extensive defects necessitate surgical intervention. However, the complex treatment procedures and high rates of graft rejection present practical limitations. Articular cartilage, a supportive connective tissue that covers the joint surfaces of bones, serves to prevent bone-to-bone friction. Water and mucin are the main components of ECM cartilage [15-16].

Glycosaminoglycan acid (GAG), chondroitin sulfate A, chondroitin sulfate C, and keratin sulfate are all polysaccharides. The fiber is made up of collagen fibers, mostly type II collagen. Due to the absence of blood and lymphatic vessels, cartilage has limited inherent repair capabilities. Articular cartilage is commonly affected by aging and joint injuries, leading to defects in the cartilage layer. Articular cartilage defects are classified as partial-thickness, full-thickness, or osteochondral. The cartilage layer is the only place where partial and full-thickness defects occur. Currently, various techniques for cartilage repair exist, such as subchondral bone drilling, cartilage transplantation, chondrocyte transplantation, and mesenchymal cell transplantation [17-19]. While these methods can offer temporary alleviation of pain and swelling linked to cartilage defects, they may also lead to the development of fibrous repair tissue or fibrocartilage that lacks the essential weight-bearing qualities of healthy cartilage. The treatment plan must include bone and cartilage repair, even if the injury is bone and cartilage-related, such as an osteochondral defect.

Currently, defect repair using an alternative approach, namely tissue engineering, is gaining popularity. Tissue engineering utilizes cells, scaffolds, and growth factors to direct the formation of bone and cartilage. Scaffolds, composed of a range of biomaterials,

serve as the foundation for housing cells and growth factors. These scaffolds are implanted into bone or cartilage defects to promote the regeneration of these tissues. Various materials such as polymers, bioglass, bioceramics, hydrogels, metals, and alloys have been employed in the development of bone and cartilage tissue. Numerous research studies have investigated the distinct attributes of metals in the realm of bone and cartilage tissue engineering [20-22].

Therefore, we discuss this trend with an emphasis on AgNPs, biomaterials, AgNPs uses, and properties of nanoparticle-based biomaterials. This research could improve our understanding of how silver-based biomaterials can be utilized to treat bone diseases more effectively, repair flaws in the bone, and reduce the risk of infection during procedures involving bone tissue.

■ BIOMATERIAL

Biomaterials are engineered materials used to treat, augment, repair, or replace body tissue functions, such as heart valves and pelvic implants. Biomaterials can also be autografts, allografts, or xenografts used as transplant materials. Biomaterials are known to have several drawbacks, including having only one function, so they cannot completely replace organ functions [23-25]. Furthermore, tissue responses to biomaterials are typically toxic and foreign body responses. Because it is used on the viscera and is bound to tissues, the materials that can be used for biomaterials are severely restricted. Biomaterials must have the following characteristics [26]. (1) Biocompatibility, biomaterials must be compatible with the body and not cause adverse reactions. (2) Non-toxicity, biomaterials must be non-toxic to body tissues and non-carcinogenic. (3) Mechanical and physical properties, to function physically and mechanically, biomaterials must have adequate physical and mechanical properties. (4) Rust and corrosion-resistant, biomaterials must be carefully chosen so that chemical reactions with body tissues do not cause corrosion in the body. (5) Design and manufacturing, biomaterials must be able to be formed or processed into various forms, be relatively inexpensive, and be readily available. While synthetic biomaterials are known to be classified into five types [27-28]. (1) Metal biomaterials, metals are widely used in the medical and health fields for implants. Biomaterials can be made from a variety of metals, including cobalt-based alloys, pure titanium and titanium alloys, and stainless steel. (2) Polymer biomaterials, polymers can be used in biomaterials because of their good elastic and plastic properties. Silicon, ultrahigh molecular weight polyethylene, acetal, polyurethane, polylactic, polyglycolic acid, nylon, and other special polymer materials are examples of polymer materials used as biomaterials. (3) Composite biomaterials. Because of their very ideal properties, namely low weight and high strength, composite-based biomaterials are widely used to replicate prosthetic limbs. (4) Ceramic biomaterials. Due to their hardness and strength, ceramics are widely used as restorative materials in dentistry, such as materials for dentures and dental cements. (5) Natural biomaterials are materials derived from living things such as animals or plants and are suitable for use as biomaterials. The use of natural materials is advantageous because natural ingredients have similarities to the structure of tissues in the body. Furthermore, it allows the binding of specific proteins and other biochemicals that can aid in tissue healing or integration [29-30]. However, these natural biomaterials are also known to have deficiencies, such as immunogenic issues, a proclivity to denature or decompose, and limited implant fabrication processes. Collagen, for example, is a natural biomaterial that is used to form connective tissues such as bones, tendons, ligaments, and skin [31-32].

■ SILVER-BASED NANOPARTICLES (AgNPS)

Silver metal particles smaller than 100 nm are known as AgNPs. Some research suggests that metal nanoparticles can be included in nanofibers to provide antibacterial properties for biomedical applications. AgNPs are the metallic nanoparticles most frequently used as bioactive polymer materials because of their increased conductivity and catalytic activity [33-35]. AgNPs were also found to be toxic to cancer cells generated from bone and to control the differentiation and multiplication of mesenchymal stem cells (MSCs)

involved in bone regeneration. Specific research on how AgNPs affect mesenchymal stem cells MSC and bone cancer cell proliferation and/or differentiation is currently few, nevertheless. Broad-spectrum and antibacterial activity against bacteria, fungi, and even viruses is one of the many benefits of AgNPs [36-39]. One reason for silver's antibacterial properties is that it can break down bacterial cell walls, interfere with cell metabolism, and prevent the formation of new microbial cells. Silver can affect cell metabolism through interactions with macromolecules in cells, including proteins and DNA [40]. Because they are friendlier for the environment and utilize fewer hazardous chemicals, AgNPs derived from plant extracts are currently more widely used.

The potential for developing AgNPs in a variety of fields, including sensors and antimicrobials, is vast. AgNPs can be used in the food industry as antimicrobial packaging films. AgNPs on the packaging are known to increase the food shelf life while also preserving taste and smell. AgNPs can be synthesized by combining 50 mL of Jatropha seed extract with 10^{-2} M $AgNO_3$ solution in various volume ratios. The solution's color changes from yellow to a reddish-brown hue, indicating the production of AgNPs, or it can be observed using a UV-vis spectrophotometer, where AgNPs have a maximum in the 400–500 nm range. It was discovered that the antibacterial activity of AgNPs synthesized with Jatropha seed extract was superior to that of commercial AgNPs products in terms of stopping the growth of Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and Gram-negative (*Escherichia coli* and *Salmonella typhi*) bacteria [37]. In medical and industrial settings, silver and other metal nanoparticles including metallic silver, silver nitrate, or silver sulfadiazine are known to be effective in treating burns, wounds, and serious bacterial infections because of their capacity to inhibit germs [41]. According to reports from other researchers, hybrids of AgNPs with amphiphilic hyperbranched macromolecules exhibit efficient antibacterial surface coatings, and silver ions and silver-based compounds are highly toxic to microbes. AgNPs have been shown to have antimicrobial properties against bacteria, viruses, and eukaryotic microorganisms. Some researchers believe that silver is an environmentally friendly metal because it does not cause allergic reactions, is non-toxic, and can be processed properly [42-44]. AgNPs have a pH of 8 and come in spherical, trigonal, and hexagonal shapes. AgNPs' discovery completely revolutionized the field of regenerative medicine and attracted significant interest because of their proven antibacterial properties and osteoinductive potential. AgNPs' discovery has completely changed the field of regenerative medicine and garnered considerable interest from scientists because of their proven antibacterial properties and osteoinductive potential. Because AgNPs have the potential to function both *in vitro* and *in vivo*, they are known to control the proliferation and differentiation of MSC, which repair bone. In addition, these AgNPs are toxic to cancer cells that grow from bone [45-49]. With further research, this biomaterial could be evolved into a new option for successful and dependable bone healing.

■ AgNPs-BASED BIOMATERIALS

The most researched and studied nanostructures produced by nanotechnology are AgNPs. AgNPs have demonstrated new characteristics and great promise for the creation of novel antimicrobial agents, drug delivery systems, coatings for biomaterials and medical devices, materials for tissue regeneration and restoration, and biomaterials [50]. The majority of silver exists in the form of compounds. In wound care management, AgNP-biomaterials are known to be non-cytotoxic and safe for patients [51]. Fig. 1 depicts the mechanism of silver nanoparticles embedded in biomaterials.

The main advantage of nanosilver-based biomaterials intended for antibacterial purposes lies in their innate ability to combat both free-floating microorganisms and those present in biofilms. The bactericidal properties of AgNPs stem from their interaction with silver cations, which bind to thiol groups in bacterial proteins, disrupting their normal functions and causing cell death [52]. Through a Trojan-horse mechanism, AgNPs first attach to the cell surface, leading to changes in permeability, disturbances in cellular respiration, and eventually penetration of the cell barrier to release silver metal ions inside the cell.

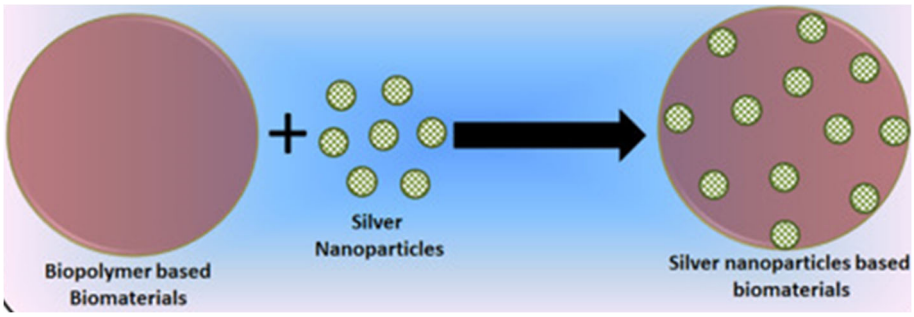

Fig 1. AgNPs embedded biomaterials

The efficacy of nanosilver-based biomaterials as potent antimicrobial agents has been empirically evaluated against a range of medically significant free-floating and surface-attached pathogenic microorganisms like bacteria, viruses, fungi, and yeasts [53-54]. The remarkable antimicrobial performance of AgNPs serves as a strong foundation for creating, enhancing, and using novel nanosilver-based biomedical products, including anti-cancer treatments, drug delivery systems, orthopedic materials, wound dressings, antiseptic sprays, and catheters. The diverse applications of AgNPs in nanotechnology, biomedicine, and environmental sectors have instigated a growing demand for the development of cost-efficient, uncomplicated, eco-friendly methods for synthesizing AgNPs [55].

■ PROPERTIES OF AgNPs BIOMATERIAL

Morphology

Characterization is a critical step in the development of materials containing AgNPs. Many studies have used transmission electron microscopy (TEM) to examine Ag dispersion. This method is highly renowned for its capacity to both measure particle size and see the dispersion of AgNPs within the substance under test. AgNP particles of 2.5 nm are clearly visible and evenly distributed throughout the polymer matrix. AgNPs' small size allows them to enter dentin tubules, which is believed to help in the inactivation of any remaining bacteria. Furthermore, AgNPs can be dispersed well in materials with small nanoparticle aggregate sizes [55].

Mechanical Properties

The mechanical properties of biomaterials containing 0–0.042% AgNP are known to be suitable for commercial composites lacking antibacterial activity. The number of microorganisms that died increased when the composite was filled with AgNPs. The amount of lactic acid produced from 0.042% AgNP was 1/3 that of commercial composites, and there were 14 colony forming units (CFU) overall for all species. Besides that, AgNP composites are beneficial for tooth restoration due to their remineralizing and antibacterial properties, particularly at low concentrations. It is known that combining AgNPs with polymeric biocompatible films can result in maximum interaction with prokaryotic cell membranes and minimal interaction with eukaryotic cell membranes. Chitlac, or lactose-modified chitosan, is one of the polymers that can be used to stabilize AgNPs at low concentrations. Indicators including stiffness, elasticity, tensile strength, and compressive strength are frequently used to gauge the mechanical strength of these polymers [56-57].

Antibacterial

In today's antibacterial applications, AgNPs are among the most commonly utilized metal nanoparticles. These AgNPs' broad intrinsic bactericidal impact against both Gram-positive and Gram-negative bacteria, along with their physicochemical characteristics, have been shown to improve their anti-pathogenic performance over silver ions [58-59]. AgNPs are known to interact with bacterial membranes and penetrate cells, causing severe disruption of cell function, structural damage, and cell death. These unique nano-related traits include high inherent antibacterial effectiveness and non-toxicity. Fig. 2 depicts the mechanism of AgNPs interaction with bacterial cells [56].

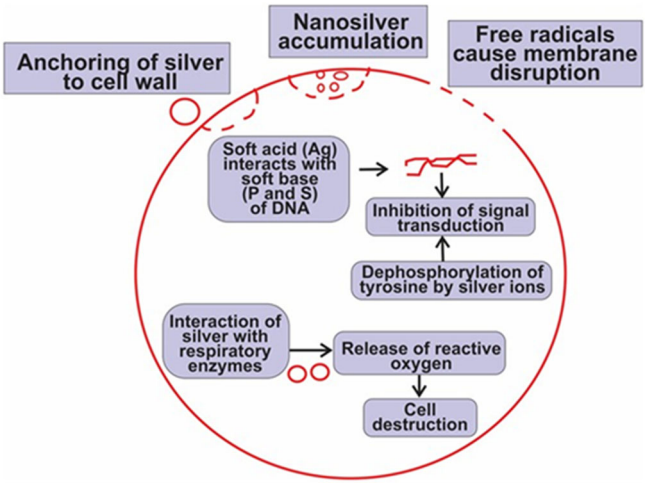

Fig 2. The mechanism of action of AgNPs against bacteria

Single metal nanoparticles have inherent antibacterial and anti-inflammatory qualities, which make the antimicrobial capabilities of these AgNPs extremely promising for usage in tissue scaffolding, wound healing, and protective clothing applications. AgNPs are found in many pharmaceutical compositions, including antibacterial clothes, burn ointments, and coatings for medical equipment and analytical uses [60-64]. Mixing antimicrobial AgNPs with natural or synthetic polymers can help minimize or even completely stop microbial contamination and the colonization process. This can be achieved if natural or synthetic polymers and antibacterial AgNPs interact effectively. Polyvinylpyrrolidone (PVP), polyethylene-glycol (PEG), and sodium citrate are a few examples of capping agents that can be employed to stabilize AgNPs against agglomeration. Size, shape, concentration, surface charge, and colloidal state are the primary physicochemical factors affecting AgNPs' antibacterial activity [63-64].

Toxicity

It is well known that AgNP stability affects toxicity. Between 0.4 to 30 g of silver is thought to be consumed daily by people from natural sources, such as food and water. AgNPs have a low toxicity and are advised for use in biomedical applications. AgNP toxicity has been observed in biological systems, including human cells, viruses, and bacteria. AgNPs can be highly successful antibacterial agents that do not harm human lung epithelial cells, mouse hepatocytes, neural cells, or healthy mammalian cells [65]. Based on the amount of AgNP that was injected, toxicity tests in a mouse ear model showed that exposure to the substance resulted in either permanent or temporary hearing loss and severe mitochondrial dysfunction. Because more retinal cells are exposed to oxidative stress, even modest amounts of AgNPs have been shown to be absorbed by these cells, resulting in notable structural alterations. The functionalization of AgNP surfaces results in surface charge changes that impact cytotoxicity, translocation to different tissues, and cellular uptake. The quantity of nanoparticles and their mode of absorption into the cell are known to be influenced by the surface charge, as determined by the zeta potential. The toxicity mechanisms involving AgNPs are shown in Fig. 3 [57].

Antivirus and Antifungal

AgNPs are known to bind to viral outer proteins, preventing them from binding and replicating. Although the antiviral mechanism of AgNPs is not fully understood, future research is possible. AgNPs have demonstrated antifungal efficacy against forty-four distinct fungus strains. AgNPs have the ability to damage the integrity of the cell membrane of *Candida albicans*, preventing the cell's growth. Consequently, fungal infections linked to oral tissues can be avoided with AgNPs. AgNPs at a concentration of 1 g/mL in resin showed potent antifungal activity without being cytotoxic [55].

■ AgNPS-BASED BIOMATERIALS APPLICATION

Dentistry

One of dentistry's main objectives is to preserve the mouth cavity, which serves as the entrance to the rest of the body. Plaque biofilm is the primary cause of dental illness. AgNPs have been added to biomaterials to stop or minimize the production of biofilms. Because of their tiny particle size and increased surface-to-volume ratio, they exhibit remarkable antibacterial action without compromising the material's mechanical qualities. AgNPs are widely used fillers in a wide range of biomaterials, where they significantly enhance certain properties. AgNP's unique characteristics make it a biomaterial with

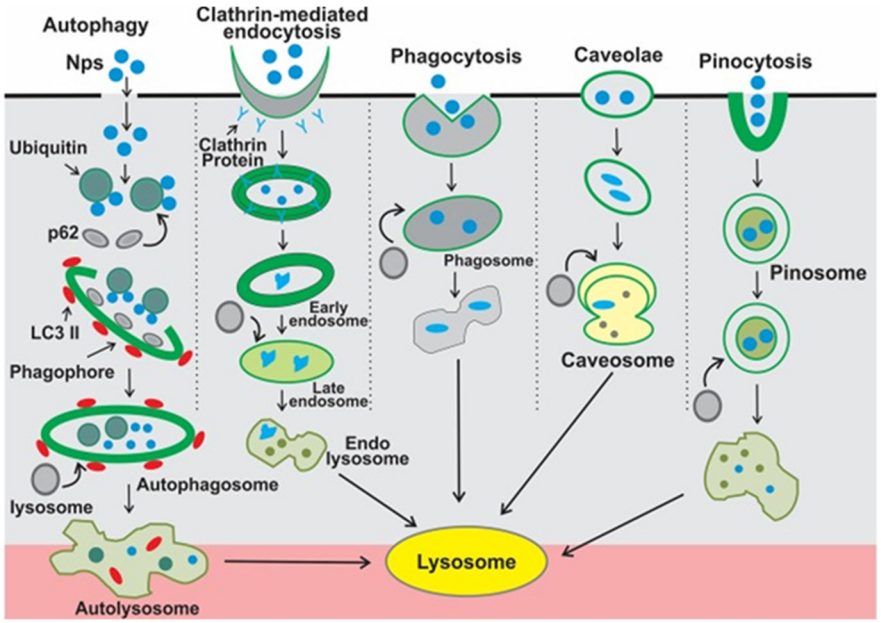

Fig 3. An illustration of potential AgNP cellular absorption techniques

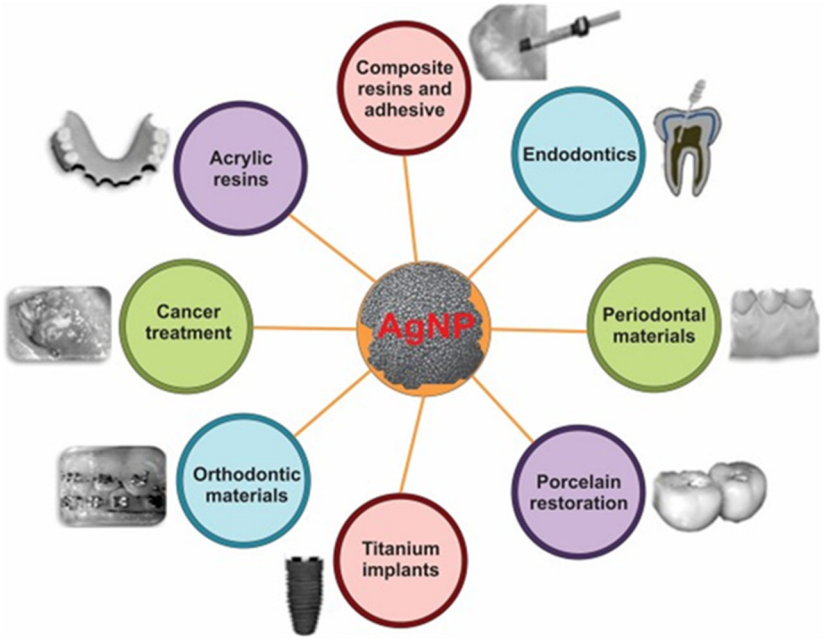

Fig 4. Biomaterials use of AgNPs

applications in orthodontics (bracket cement), endodontics, periodontics, implant dentistry (titanium dental implants), prosthetic dentistry (acrylic and porcelain resin), oral surgery, and restoration [61,66-67], which are detailed in Fig. 4. Despite the fact that a variety of biomaterials have been employed to treat dental conditions, the expected outcomes have not always been achieved due to limited material characteristics.

Wound Dressing

Many composite dressings can benefit from the use of silver, an anti-microbial substance that is extensively used and effective against infectious diseases that cause wounds to heal slowly [68-71]. Wound dressings containing silver release different amounts of silver ions depending on the form, quantity, and combination of

silver, such as silver sulfadiazine, ionic AgNPs with scaffolds, and nanofibers containing AgNPs [72-76]. When it comes to burn wounds, *Pseudomonas* spp. exhibit bacterial resistance to silver sulfadiazine and silver nitrate, despite the fact that silver's multifunctional action has a low risk of resistance in infected wounds/biofilm formation. Additionally, depending on the pace at which silver ions are released, the addition of silver to different dressings might also affect the overall antibacterial impact. Certain methicillin-resistant strains of *S. aureus*, *P. aeruginosa*, and *E. coli* were shown to be less sensitive to the antimicrobial dressings Urgotul SSD®, Bactigras®, Acticoat®, and Askina Calgitrol Ag®, as well as Aquacel Ag®. Moreover, silver affects host proteins in addition to bacteria. Therefore, the impact on the host tissue will be larger and recovery may be slowed if there are very few bacteria at the wound site.

Excretions from wounds containing more than 1 mg/L of silver react and can produce transient skin discoloration. However, using low amounts of silver may lead to bacterial resistance. Since *S. aureus* and *P. aeruginosa* are known to build biofilms in wound settings that are persistently infected, these dressings containing silver may provide them with resistance. In addition to reducing or eliminating infection from outside sources, this silver dressing is said to be effective in eliminating germs solely in the area of the wound being bandaged [72-73]. Chronic wounds that are infected and slowly healing have significant exudate, bacterial resistance, biofilm formation, extended inflammation, and inadequate systemic antimicrobial efficacy. Composite dressings (film and wafer) of polyox/carrageenan (POL-CAR) and polyox/sodium alginate (POL-SA) loaded with streptomycin (STP) and diclofenac (DLF) have been found in several trials to exhibit antibacterial action against bacteria in infected wounds chronic with *S. aureus*, *P. aeruginosa*, and *E. coli*, respectively. According to their findings, composite polymer-based dressings with DLF and STP seemed to suppress all three bacterial strains considerably better than commercial dressings with silver. DLF's non-conventional antibacterial action resulted in a greater minimum inhibitory concentration (MIC) than STP. Dressings that contain STP and DLF are very efficient against *S. aureus*, *P. aeruginosa*, and *E. coli*. The POL-SA dressing fared better against all three types of bacteria than the POL-CAR formulation, while the DLF and STP treatments surpassed the silver-based dressings. Nonetheless, the film exhibits superior antibacterial activity compared to silver wafers and dressings [77-78]. Since DLF has an anti-inflammatory effect, it may also help reduce swelling and pain associated with injuries. Additionally, STP's antibacterial action may help lower bacterial infections. However, further *in vivo* animal studies and *in vitro* cell culture research are needed for this (for cell viability and migration/proliferation).

■ BONE TISSUE ENGINEERING

Currently, tissue engineering offers hope in the fields of regenerative and remedial medicine. Utilizing the right models and technology, a multidisciplinary approach to biomedical and regenerative medicine applications is created by combining materials engineering, biological sciences, and computer modelling. The focus of research has switched from tissue replacement to tissue regeneration in order to address the growing need for tissue repair. Using a combination of scaffolds, growth signals (growth factors), and germ cells, tissue engineering aims to replace, repair, and regenerate damaged target tissues [79-82]. To determine if novel biomaterials are suitable for a certain application, *in vivo* research is necessary. For bone and cartilage tissue engineering research, the most common animals employed are mice, rats, sheep, pigs, rabbits, and primates. The best models are monkeys since they are anatomically and physiologically similar to humans. However, the broad use of monkeys is challenging because of their high cost and limited supply. Large animals, like goats and pigs, are expensive and challenging to feed, thus they are rarely utilized. Combining histological examination with imaging data can show new bone production, such as total bone volume (TBV) and bone mineral density (BMD).

Moreover, the state of tissue regeneration and the development of new bone, cartilage, and other tissues can be demonstrated by a few widely used staining

techniques such as hematoxylin and eosin (HE) stain and safranin stain. Since they are inexpensive and simple to work on, rats and mice are frequently employed. Because copper has strong anti-inflammatory and biocompatibility qualities, it is well known that copper-containing scaffolds implanted in rodents have good osteogenic and angiogenic abilities. Many studies have been conducted on hydroxyapatite doped with cations such as Cu^{2+}, Sr^{2+}, Ag^{+}, Mg^{2+}, and Zn^{2+}, enhancing bioceramic materials' antibacterial capabilities. The potent antibacterial action of Ag^{+} ions against bacteria, fungi, and many viruses makes them attractive dopants [83]. Zn^{2+}, a trace element, has been shown to exhibit antibacterial activity against both Gram-positive and Gram-negative bacteria as well as fungi. It has an impact on bone physiology as well. However, these encouraging characteristics are also influenced by the toxicity of metal ions. In this instance, n-Hap can benefit from the addition of metal ions to increase the antibacterial activity of the materials with a minor impact on their biocompatibility. Ag^{+} and Zn^{2+} ions have been shown to cause less cellular stress than other metal ions when utilized as dopant elements in biominerals. The biocompatibility and antibacterial activity of metal ion-doped n-Hap have been studied in multiple investigations. A narrow shareability range between the two features and inconsistent results in osteoblast cell lines suggest a lack of understanding of the cellular response to doped materials. Therefore, enhancing n-Hap's biological potency still poses a considerable problem of striking a compromise between biocompatibility and antibacterial activity [83-84].

■ SILVER NANOPARTICLES FOR BONE HEALING

Bone illnesses include cancer, degenerative and hereditary conditions, infectious infections, and bone fractures that harm millions of individuals globally each year. Because of the high morbidity of related infections, opportunistic contamination and colonization of orthopedic implants is a significant concerns in bone tissue replacement schemes. Bone is an active tissue that uses intricate and intrinsic processes for bone remodeling to grow and repair itself [84]. Bone grafts are typically utilized to replace or treat serious abnormalities, such as genetic illnesses, cancers, or injuries, that permanently alter bone tissue. High levels of inflammation, implant loss, and bone degradation are typically linked to orthopedic and bone implant infections [85-86]. Previous studies have demonstrated that AgNPs spontaneously promote bone mineralization and the differentiation of MC3T3-1 pre-osteoblast cells. These days, prostheses plated with silver offer a novel way to stop common infections linked to trauma and tumors. Nevertheless, there hasn't been any published clinical research comparing the long-term clinical effects of nanosilver-coated implants for revision arthroplasty. Bone's capacity to mend itself is compromised when bacterial activity takes place on injured bone. AgNPs have a wider range of intrinsic antibacterial activity when compared to traditional antibiotics. The peculiar occurrence of bacterial resistance to AgNP activity highlights the synergistic action of nanosilver's bactericidal mechanism [87].

In the case of antibiotic-resistant bacteria, such as methicillin-resistant *S. aureus*, AgNPs have been shown to inhibit or interfere with the development of biofilms or mature biofilms, respectively. AgNPs have been demonstrated to impede or prevent the formation of mature biofilms or biofilm development in antibiotic-resistant bacteria, such as methicillin-resistant *S. aureus*. AgNPs are frequently employed in bone replacement treatments as doping materials for synthetic and bioinspired bone scaffolds. Inducing antibacterial characteristics in HA coatings has been demonstrated to be feasible by a number of experimental approaches, including laser-assisted deposition, magnetron sputtering, ion beam-assisted deposition, sol-gel technology, electrochemical deposition, and micro-arc oxidation. Certain studies claim that because of a micro-galvanic interaction created between the implanted AgNPs and the titanium substrate, AgNP-embedded titanium offers superior antibacterial properties and great osteoblast compatibility. To provide innovative and functionally improved biomaterials for orthopedic applications, numerous studies examined the viability and therapeutic potential of adding AgNPs to acrylic cements. While earlier research examined a variety of

acrylics modified with AgNPs, most of that work was constrained by the incomplete analysis of crucial material attributes and mechanical qualities [85]. In previous study, AgNP-embedded titanium has demonstrated remarkable antibacterial capabilities and great osteoblast compatibility. By encouraging osteogenesis and MSC proliferation, antimicrobial AgNPs can enhance fracture healing [55,87-88].

■ AgNPs FOR OTHER MEDICAL APPLICATIONS

New biomedical procedures are developed and applied with the help of AgNPs' distinct physiochemical characteristics and biofunctional features, which include anti-inflammatory, antiplatelet, antifungal, antivirus, anti-angiogenesis, and antibacterial activities. AgNPs have shown encouraging anticancer effects on a range of human cancer cells, such as MDA-MB-231 breast cancer cells, IMR-90 fibroblasts from the lungs, U251 tumor cells, and endothelial cells. These effects are currently the subject of extensive research. AgNPs can naturally bind to and readily enter mammalian cells through internalization processes driven by energy. AgNPs' unique fluorescence is another enticing feature that makes them a good choice for dosage escalation and detection in X-ray irradiation systems [55-56]. The plasmonic structures known as AgNPs have the ability to both disperse and absorb light in a particular region. The light absorbed by the AgNPs can be employed for selective hyperthermia, while the dispersed light they release after selectively absorbing into cancer cells can be used for imaging.

Considering more than 17.7 million fatalities from 2015 to the present, cardiovascular diseases (CVDs) are the primary cause of death globally [53]. Contradictory results have been obtained in recent research examining the impact of AgNPs on different cell types present in intricate vascular systems. However, the information acquired might shed light on the possible advantages of AgNPs for physiological and pathological phases pertaining to the cardiovascular system, which could aid in the creation of novel and targeted molecular treatments for vascular tone, vasopermeability, and angiogenesis, which AgNP toxicity may be influenced by cardiovascular conditions like hypertension. The first cardiovascular medical equipment to be treated with silver was a prosthetic silicone cardiac valve coated in silver, which was intended to lower inflammation and prevent bacterial infections connected with the valve. The human eye is a complicated organ with outstanding vascularization and sensitivity that is susceptible to microbial contamination under the correct temperature and humidity circumstances. Compounds and materials based on nanosilver have demonstrated encouraging promise for creating new treatments and enhancing the effectiveness of infections connected to the eyes. AgNPs coated with an indicator of calcium have been demonstrated to cause less harm to cells in the retina and can be utilized in an animal model of mice for retinal imaging [55]. Before using AgNP-containing nanomaterials as an improved class of antibacterial substances for ocular applications, it is vital to explore their bactericidal impact further. AgNPs have the potential to be a novel nanostructured platform for the diagnosis and treatment of cancer. AgNPs are attractive for essential multichannel and tumor resistance control tactics in addition to anti-infection strategies due to their broad-spectrum bioactivity.

Under the right temperature and humidity conditions, the human eye is a complex organ with impressive vascularization and innervation that is easily exposed to microbial contamination. Nanosilver-based compounds and materials have shown promising potential for developing novel therapies and improving the performance of eye-related infection conditions. AgNPs coated with a calcium indicator were shown to have less damage to retinal cells and can be used for retinal imaging in a mouse animal model [55]. The bactericidal effect of AgNP-containing nanomaterials is an important aspect that should be investigated further before they are used as a better class of antibacterial agents for ocular applications. AgNPs can be successfully used as a new nanostructured platform for cancer diagnostics and treatment. AgNPs' broad-spectrum bioactivity makes them appealing not only for anti-infection strategies but also for critical multidrug and tumor resistance management approaches. Global public health is greatly concerned about malaria, one of

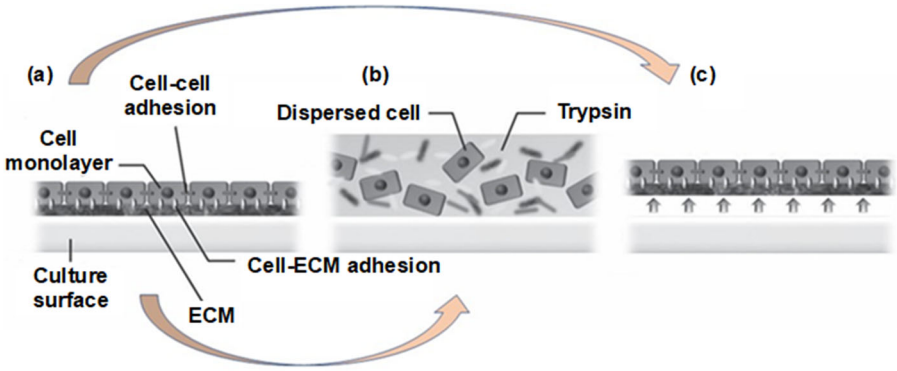

Fig 5. Cell sheet technology diagram. (a) The culture surface has confluent cells. (b) ECM and additional protein surfaces are broken down by trypsin. As a result, cells disperse and proteins critical to cell function are lost. (c) When the adhesion between the surfaces of the cell cultures is selectively removed, the confluent cells can be separated in sheets

the most prevalent infections in both tropical and subtropical areas. It has been demonstrated that AgNPs exhibit strong anti-malarial effects towards the parasite *Plasmodium falciparum* and its female vector, the *Anopheles* mosquito [55]. Global malaria control and nanotechnology-derived medicines have a strong foundation thanks to the inherent anti-plasmodial characteristics of chemicals and materials based on nanosilver.

Cell sheet

The cells form a confluent monolayer with strong adhesion between them on the surface of the culture when they reach the junction. Adhesion proteins are generally broken down nonspecifically when cells are harvested using enzymes like trypsin, which causes individual cells to become dispersed. The cells shed in the form of cell sheets if the adhesion between them and the cell culture surface is specifically eliminated (Fig. 5). It has been claimed that various cell sheet manufacturing processes exist, even though temperature-responsive polymer-based approaches are leading the development and application of cell sheet technologies [89-91]. Surface modification-based methods, methods that do not include surface modification, and temperature-responsive polymer-based methods are the three groups into which cell sheet manufacturing technologies fall. They all have benefits and uses of their own.

Although the usefulness of cell sheets prompted the creation of cell sheet systems for a range of uses, such as the fabrication of bio-actuators and organ modelling, this review concentrates on basic tissue engineering research. While basic research on cell sheet technology has been carried out and documented for the improvement of tissue engineering, clinical research is discussing strategies for preparing and enhancing cell sheet functionality [89-95]. Please take note that most of the studies assessed for articles with temperature-responsive culture surfaces included improving the function of cell sheets. Since perfusion vascular structure technologies have the potential to be a major technological advancement in cell sheet technology, individual trials toward achieving this goal were examined. Each section is outlined in Fig. 5, which also gives a general overview of cell sheet technology [89].

■ CONCLUSIONS AND FUTURE PERSPECTIVES

We give a summary of the properties of AgNPs, their biomaterials, and their uses in the tissue engineering of cartilage and bone. The scaffolds' degradation behavior may be impacted by the incorporation of silver, which can also increase the scaffolds' porosity, mechanical strength, and cross-linking. AgNPs are helpful for bone, cartilage, and vascular repair, according to studies conducted in living animals. Future studies should examine the precise mechanism of the AgNPs-induced increase in osteogenic and chondrogenic properties. Extensive research should also be conducted to precisely establish the size and structure of AgNPs used in tissue engineering, be it

cartilage, bone, or other specific tissues. Future developments in AgNP-based biomaterials will involve improving efficiency and safety, applying nanomedical technology, incorporating nanotechnology into biomaterial structures, and creating smart and regenerative implants. With these advancements, it is believed that the treatment of bone disorders and injuries would become more effective, individualized, and long-term.

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■ CONFLICT OF INTEREST

The authors declare that they have no known competing interests that could have influenced the literature reported in this review.

■ AUTHOR CONTRIBUTIONS

Nasmi Herlina Sari: Conceptualization, methodology, writing – original draft, review, editing, supervision, and funding acquisition. Muhammad Zaidan Fadhlurrohman Rivlan, Suteja: Resources, investigation, data curation, and visualization. Hamsu Kardiyan, Senthil Muthu Kumar Thiagamani: Data curation, review, and editing. All authors approved the final version of this manuscript.

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