EFFECT OF METALLIC NANOPARTICLES ON WOUND HEALING ACTIVITY

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***Abstract*: In spite of various treatments available, still wound healing is a big challenge because of increasing number of people suffering from wounds and the cost of wound care. Nanotechnology is one such treatment option which is grabbing greater attention nowadays. Development in the field of nanotechnology and its applications to the field of medicines and pharmaceuticals has transformed the twentieth century. Nanotechnology through the use of nanomaterials unlocked a new chapter in wound healing treatment suggesting solutions for the acceleration of wound healing, as well as demonstrating distinctive properties as bactericidal agents. In these nanomaterials metallic nanoparticles are increasingly being used due to their favourable effect on accelerating wound healing, as well as treating and preventing bacterial infections. Other advantages include: ease of use, less frequent dressing changes and a persistent moist wound environment. In this article we are going to explore the effect of metallic nanoparticles on wound healing activity.**

***Keywords*: Wound healing; Nanotechnology; Nanomaterials; Metallic nanoparticles; Bi metallic nanoparticles; Trimetallic nanoparticles.**

Introduction

A wound is defined as damage or destruction to the normal anatomical structure and function. This can range from a simple break in the epithelial integrity of the skin or it can be deeper, prolong into subcutaneous tissue with damage to other structures such as tendons, muscles, vessels, nerves, parenchymal organs as well as bone [1]. Wounds can appear from pathological processes that start externally or internally within the involved organ. They can have an unintended or intentional aetiology or they can be the result of a disease process. Wounding, irrespective of the cause and whatever the form, injure the tissue and interrupt the local environment within it [1].

Wounds can be of acute or chronic based on their time taken to heal [2]. Many factors play role in formation of chronic wounds such as high levels of inflammatory mediators, wound infection, formation of biofilm, hypoxia (common chronic conditions cardiovascular disease, smoking, pulmonary disease and peripheral vascular disease results in hypoxia), drugs (inotropes, steroids, NSAIDs, diabetes mellitus, elderly persons, poor nutrition with low protein level can delay wound healing process [3].

The normal wound healing is a dynamic and complex development involving a sequence of co-ordinated events, including bleeding, coagulation, initiation of an acute inflammatory response to the initial injury, regeneration, migration and multiplication of connective tissue and parenchyma cells, as well as synthesis of extracellular matrix proteins, remodelling of new parenchyma and connective tissue and collagen deposition. Finally, increasing the wound strength takes place in an ordered manner and culminates in the repair of severed tissue

Wound healing is a still challenge because of increasing number of people suffering from wounds and the cost of wound care.

As per studies, a 2018 retrospective analysis of Medicare beneficiaries conclude that *8.2 million people had wounds with or without infections. Medicare cost estimates for acute and chronic wound therapy ranged from $28.1 billion to $96.8 billion [4].

The global wound care market size was valued at USD 19.83 billion in 2020 and is expected to do more at a compound annual growth rate (CAGR) of 4.1% from 2021 to 2028 [5].

The understanding and therapy strategies for wound healing have gone through a great revolution [2].

The various methods are used in treating wounds such as debridement, skin substitutes, growth factors, wound dressings, gene therapy, stem cell therapy, antiseptics, antibacterial agents [2], traditional therapy-based herbal and animal derived compounds, living organisms [6], electrical field stimulation [1].

Another treatment option which grabbed greater attention is nanotechnology.

Nanotechnology is the study of extremely small structures. The prefix “nano” is a Greek word which Refer “dwarf”. The word “nano” refers to very small scale or miniature size [7]. Nanoparticles (NPs), usually ranging in dimension from 1-100 nanometres (nm), have exclusive properties from their bulk equivalent. They possess uncommon physicochemical, optical and biological properties, which can be manipulated suitable for desired applications [8]. They are contributing advantages not possessed by larger particles, such as increased surface to volume ratio or improved magnetic properties [9]. Advancement in the field of nanotechnology and its utilization in the field of medicines and pharmaceuticals has Remodel the twentieth century [8].

Nanotechnology through the utilization of nanomaterials opened a new chapter in wound healing therapy proposing solutions for the acceleration of wound healing, as well as presenting distinctive properties as bactericidal agents [10].
The drug itself may be developed at a nanoscale such that it can work as its own "carrier" or nanomaterials may be used as drug delivery vehicles [10].
The main breed of nanomaterials used for wound therapy are represented by nanoparticles, nanocomposites, coatings and scaffolds. (Are represented in figure).
1) Nanoparticles – a) Inorganic – metal and non-metal
b) Organic – Non polymeric and polymeric
2) Nanocomposites – porous materials, colloids, copolymers, gels
3) Coatings and scaffolds – Hydrogels, nanofibers, films and coatings [11].
In these nanomaterials metallic nanoparticles are increasingly being used due to their beneficial effect on accelerating wound healing, as well as treating and preventing bacterial infections. Other benefits include: ease of use, less frequent dressing changes and a persistent moist wound environment [11].

This review explains about the effect of metallic nanoparticles on wound healing activity.

METALLIC NANOPARTICLES

Depending on the number of metals and metal oxide
Metallic NPs are divided into monometallic, bimetallic, trimetallic and quadrometallic types. In between these, bi-, tri-, and multimetallic NPs have attracted the greatest interest due to their enhanced catalytic properties and favourable characteristics compared with monometallic NPs [12].

MONOMETALLIC NANOPARTICLES

Monometallic NPs occupy only one type of metal with particular chemical and physical properties [12]. Metal-based NPs that have been reported for antimicrobial activity and wound healing activity are silver NPs (AgNPs), gold NPs (AuNPs), copper NPs (CuNPs), Moreover metal oxide NPs such as zinc oxide NPs (ZnO NPs), titanium dioxide (TiO_2), cerium oxide (CeO_2), yttrium oxide (Y_2O_3) etc [13].

Silver nanoparticles: - Ag has been known as a material of wound healing from the long past historical ages. In the modern times, AgNPs have been used in wound dressings due to their extraordinary features of anti-microbial activity and wound healing property along with large surface area to volume ratio [14].
As per literature reports the silver nanoparticles can regulate anti-inflammatory cytokine release and aid in rapid wound closure without increasing scarring. They also play role in epidermal re-epithelization through the proliferation of keratinocytes [10].
The accurate mechanism of action of silver on the microbes is still unexplored. Though the likely mechanism of action of metallic silver, silver ions, and silver nanoparticles has been proposed according to the morphological and structural changes found in the bacterial cells.
Mechanism of Action of Silver Nanoparticles (AgNP). The silver nanoparticles exhibit efficient antimicrobial properties compared to other silver compounds because of their large surface area. The larger surface morphology allows better contact with microorganisms. AgNP penetrate inside the cell by interacting with cell membrane. Bacterial cell membrane consists proteins, which include sulphur. AgNP interact with these proteins, also with the phosphorus containing compounds, like DNA. After entry into the bacterial cell it forms a low molecular weight region in the centre of the bacteria to which the bacteria conglomerate, thus preserving the DNA from the silver ions. The AgNP interact with the respiratory chain and cell division finally leading to cell death [15].
AgNPs are more powerful at lower concentrations, thus decreasing their toxicity. However, a report by *Szmyd et al.* shown that higher concentrations of AgNPs reduce keratinocyte viability, metabolism, as well as migration and differentiation of these cells, through the activation of caspase 3 and 7 (proteases involved in programmed cell death) and dose-dependent DNA damage [16].
For the purpose of reducing side effects, silver nanoparticles can be given in low doses along with antimicrobial drugs to achieve enhanced efficiency.
In a recent study by *Ahmadi et.al* demonstrated that AgNPs combined with tetracycline significantly decreased bacterial load both in superficial and deep tissue layers in a mouse model, thus accelerating healing [17]. These outcomes encourage the association of AgNPs with traditional antibacterial agents or dressings, in aiming to more efficiently treat infected wounds.
In a recent study performed in vivo on dogs, *Mihai et.al* demonstrated that silver impregnated coatings ensures faster wound healing and avoid microbial colonization on site [18].
In addition, silver nanoparticles were utilized for various clinical trials in the therapy of wounds, principally for burns and chronic wounds (i.e., diabetic ulcers). At present, there are few commercially available dressings containing AgNPs.
One such example is Acticoat©-wound dressing containing AgNPs with average size of 15 nm.
The proved properties of Acticoat© are: wound healing, infection reduction on the site of the wound, and pain reduction, aspects observed in most tested patients. Although, this coating is still under examination for the therapy of burns. Recent clinical trials concluded that Acticoat© may be efficient in avoiding infections in burns when applied together with silver sulphadiazine and chlorhexidine digluconate cream [11]. Silver and other non-antibiotic treatments were forgotten when penicillin and other antibiotics were discovered but, today, silver has acquired significant attention because of the emergence of antibiotic-resistant strains and its low tendency to develop resistance. Due to inherent therapeutic properties and multi-site action, silver nanoparticles show a broad-spectrum antibacterial capability against many micro-organisms and demonstrate a greater potential to overcome the emerging

issues in the area of microbial resistance in different applications and, in particular, in therapeutically enhanced healthcare. Silver nanoparticles (AgNPs) have shown greater potential in different applications, such as in detection and diagnosis, drug delivery, for coating of biomaterials and devices, for novel antimicrobial agents, and in regeneration materials.
Promising results have also been reported for AgNPs-incorporated biomaterials, such as modified cotton, bacterial cellulose, chitosan, and sodium alginate [19].

Gold nanoparticles:-
Gold nanoparticles (AuNPs) are of best option when it comes to wound therapy because of their chemical stability and capacity of absorbing near infrared light, while, at the same time, being relatively easy to synthetize. Additionally, by changing the surface plasmon resonance, AuNP gels exhibit thermo responsiveness, as described by *Arafa et al.*, who demonstrated their in vitro and in vivo antibacterial and healing properties, backed by histopathologic examinations [20].
Also, the study by *Sherwani et al.* demonstrated the ability of Au NPs to heal wounds [21].
AuNPs exhibit bactericidal and bacteriostatic properties by directly targeting the bacterial cell wall, or they can bind to bacterial DNA, blocking the double-helix from uncoiling during replication or transcription. As a result, they can prevent multidrug-resistant pathogens, such as Staphylococcus aureus and Pseudomonas aeruginosa [11].
One more possible mechanism of Au NPs is that they can permeate bacterial cells and alter bacterial membrane potential, which results in the inhibition of bacterial energy metabolism. Further, it also inhibits the activity of ATP synthase enzyme, which results in bacterial cell death. Moreover, Au NPs act as antioxidants by inhibiting the bacteria from producing reactive oxygen species which further help in the wound-healing process.
Au NPs can effortlessly be combined and cross-linked with collagen, gelatin, and chitosan for better wound-healing effects. This process of functionalization assists the Au NPs to gain properties such as biocompatibility and biodegradability [22].
Lorena Maria Cucci et,al did functionalization of gold nanoparticles with angiogenin for improved wound healing applications[23].
In such regard a study conducted by *Volkova et al.* demonstrated that Au NPs combined with cryopreserved human fibroblasts (CrHFC-AuNP) applied topically to burn wounds resulted in an increased overall healing rate with reduced inflammatory phase and enhanced deposition of collagen [24].

Copper nanoparticles:-
In recent times copper nanoparticles (CuNPs) have grabbed greater research interest. As copper (Cu) plays a complex role in various cells, it modulates several cytokines and growth factor mechanisms of action, and is essentially involved in all stages of the wound healing process. Cu is popular for its antimicrobial activity and has long been known to play a direct role in angiogenesis. It has also been proven that copper has greater role in wound healing process by regulating the expression level of 84 genes which are associated with angiogenesis and wound repair. Studies have shown that copper nanoparticles (CuNP) can stimulate angiogenesis by affecting the expression of hypoxia inducible factor (HIF-1a) and regulation of the secretion of vascular endothelial growth factor (VEGF) [7e9].
Various studies regarding wound healing potential of copper nanoparticles are conducted as *Sanaz Alzadeh et.al.* examined the potential therapeutic effect of various CuNPs concentrations (1 mM, 10 mM, 100 mM, 1 mM, and 10 mM) and sizes (20 nm, 40 nm, 80 nm) in wound healing. Overall, their results have indicated that the 1 mM concentration of 80 nm CuNPs is a promising NP for wound healing applications without adverse side effects[25].
And also, copper nanoparticles can be used in wound dressings to improve wound healing activity a study conducted by by *Ensieh Ghasemian Lemraski et.al* showed improved wound healing when copper nanoparticles are incorporated in chitosan/polyvinyl alcohol wound dressings [26].
Even a studies on the wound healing properties of copper nanoparticles as a function of physicochemical parameters were studied. A study done by A.*A.Rakhmetova et.al* examined the wound healing potential and physicochemical characteristics of copper nanoparticles which are modified with a variety of agents. The ointment of modified copper nanoparticles exhibited wound healing behavior that varies in effectiveness depending on their physicochemical parameters. Copper oxide nanoparticles (modified with air), having a particle size of 119 nm and crystalline copper content of ~0.5%, and copper nanoparticles (modified with oxygen), having a particle size of 103 nm and crystalline copper content of 96%, demonstrated the maximum specific rate of wound adhesion[27].

Zinc oxide nanoparticle:-
Several factors determine the effect of zinc oxide on wound healing as zinc ions are biogenic, part of many enzymes and indirectly control the activity of more than 200 enzymes and exhibit immunomodulatory properties.
A small part of zinc ions produced by ZnO NPs have high wound skin permeability, antioxidant properties, promote the regeneration of damaged tissues by activating collagen synthesis and regulate the redox imbalance during the burn disease due to tissue hypoxia in the wound [28].
Zinc oxide nanoparticles (ZnONPs) exhibit a definitive antibacterial agent, by causing bacterial cell membrane perforations. In addition, they are also utilized in hydrogel-based wound dressings where they enhance overall contact time, promote keratinocyte migration, thus upgrade the re-epithelialization [11].
In recent study by *Carol M Cleetus et.al* concluded that ZnO NP and alginate-based 3D printed lattice structures provide a viable alternative to existing chronic wound healing treatment options [29].
Aiming to provide a better treatment for burn wounds
New nanocomposites were developed by *Nina Melnikova et.al* using bacterial cellulose (BC) and betulin diphosphate (BDP) pre-impregnated into the surface of zinc oxide nanoparticles (ZnO NPs) for the production of wound dressings and the study of their

biological activity on a rats with thermal burn model. The results showed effective wound healing with BC-ZnONPs-BDP nanocomposites [28].

Further, the ZnO nanoparticles antibacterial and antibiofilm effects were evaluated by *Sajjad Mohsin I. Rayyif et.al* and the results found that ZnO-coated dressings inhibit biofilm development of main wound pathogens and represent efficient candidates for developing bioactive dressings to fight chronic wounds [30].

ZnO NPs are found to reduce UV photodamage to the skin, by their antioxidant property and preventing DNA damage in humans. The incorporation of ZnO NPs can aid photo protect damaged skin from UV irradiation during wound healing.

Cheng-Chih Lin, et.al showed that by incorporating as little as 1.0 wt % spherical ZnO NPs to the barrier film spray leads to as high as 80% of Hs68 cells to survive after 1 h UV irradiation, compared to 55% without photoprotection. Researchers have also attempted to create core-shell nanocomposites by merging two metal [11].

In this regard, *Batool et.al* showed the healing efficiency of mouse wound using ZnO-NP/silica gel (ZnO-NP/SG) dressings due to its extensive evidence on healing property. The microscopic examination of the wound area is decreased remarkably using ZnO-NP/SG-30 ppm dressings within 11 days of observation as compared to ZnO-NPs of the control sample. These findings elaborated that as-synthesized dressing enhanced the skin repairing on the wound surface and support its usage in the future [32].

Reihaneh Haghniaz et.al showed that ZnFe2O4 NPs have promising antibacterial properties and desirable biocompatibility to promote wound healing. Hence, the optimized ZnFe2O4 NPs can be used as an alternative therapeutic agent against drug-resistance microbial pathogens and also as a bandage to enhance burn-wound healing in near future [33].

BIMETALLIC NANOPARTICLES

Bimetallic NPs are formed via the integration of two different types of metal atoms to form a single nano metric material with varying structures, morphologies, and properties. Bimetallic NPs can be formed by choosing the suitable metal precursors to attain the desired shape, size, structure, and morphology according to the configuration of atoms, and at last this results in the formation of alloy, core–shell, and aggregated nanoparticle types. Bimetallic NPs, such as Ag/Au, Ag/Cu, Au/Pt, Au/Pd, Ag/Fe, Fe/Pt, Cu/Zn, Cu/Ni, Au/CuS, and Fe_3SO_4/Ag NPs, have extraordinary surface activities. More over, bimetallic oxide NPs, such as MgO/ZnO, CuO/ZnO, and Fe_3O_4/ZnO NPs, because of tensile strain and synergism between the constituent metals, often show unique antibacterial performance [12].

Ag/Au bimetallic nanoparticle

AuNPs and AgNPs are the most extensively used nanomaterials in biomedical applications. AuNPs show chemical stability and biocompatibility, while AgNPs exhibit good antimicrobial effects. However, AgNPs are less stable under identical biological and chemical conditions than AuNPs, and may show toxic effects in biological systems. Therefore, a combination of gold and silver in one bimetallic nanostructure helps to obtain a new set of properties resulting from both metals [34].

To explore the wound healing potential of bimetallic nanoparticles,

A test carried out by *Piotr Orlowski et.al* on bimetallic Au@AgNPs sized 30 nm modified with selected flavonoid and non-flavonoid compounds for wound healing applications, showed the results that Tannic acid-modified Au@AgNPs promoted epithelial-to-mesenchymal transition (EMT) – like re-epithelialization, at the same time other polyphenol modifications of Au@AgNPs acted through proliferation and wound closure [34].

ZnO/Ag bimetallic nanoparticle

A study suggests that ZnO/Ag bimetallic nanoparticles can be used in wound dressings to treat antibacterial resistance and infection. *Min Hu et.al* developed a new wound dressing that combines ZnONPs and AgNPs to produce an improved antimicrobial activity against both Gram-negative and Grampositive bacteria. The synthesized ZnO/Ag/PVP/PCL nanofibres exhibited the outstanding gaseous exchange ability, a solid haemostatic ability and a low weight loss rate. Simultaneously, the bimetallic nanofibres could synergistically improve the antibacterial effect. These preliminary findings provide a new candidate in treating the increasing antibacterial resistance and infection [35].

Ag/Cu bimetallic nanoparticle

G. Mamatha et.al. developed the nanocomposite cotton fabrics where bimetallic nanoparticles were in situ generated in cotton fabrics of using aloevera extract as reducing agent. The nanocomposite cotton fabrics have exhibited satisfactory antibacterial activity against five bacteria. These fabrics can be used for dressing, wound healing, packaging, and medical applications [36].

Using a chemical reduction method *Jaehee Jang et.al* incorporated silver (Ag) and copper (Cu) bimetallic nanoparticles on a GO surface (Ag/Cu/GO) and their antimicrobial effects against various bacterial species were demonstrated and by the topical application of Ag/Cu/GO, a biofilm-infected skin wound of a small animal model healed rapidly and efficiently. So, the study suggests that the Ag/Cu/GO nanocomposites could be used to effectively eradicate antibiotic-resistant bacteria and treat diseases in the skin or wound due to bacterial infections and biofilm formation [37].

TRIMETALLIC NANOPARTICLES

Trimetallic NPs are prepared from three different metals to decrease metal consumption, atomic order, and to fine-tune the size and morphology of these NPs. They show greater catalytic selectivity/activity and efficiency in various applications, such as biomedical, antimicrobial, catalytic, active food packaging, and sensing applications. As it contains three metals, there are some chances for different structures and morphologies, such as core–shell, mixed structure, subcluster segregated, and multishell. For better catalytic effect, trimetallic NPs were developed as alloys and intermetallic NPs by transforming the atomic distribution and surface compositions of different metals. Trimetallic NPs offer innovative physicochemical properties because of their combined or multifunctional effects for various promising applications than monometallic and bimetallic nanomaterials [11].

To date not much of the studies available on the wound healing potential of the trimetallic nanoparticles but the various studies are going on the antimicrobial potentials of trimetallic nanoparticles is studied. A study done by *Navneet Yadav et.al* on antimicrobial activity of trimetallic Au/Pt/Ag nanoparticle based nanofluids showed that trimetallic nanofluid has better antimicrobial activity than monometallic Au and bimetallic Au/Pt nanofluid at a very low concentration. These outcome of trimetallic nanofluids can be made use in the field of many applications such as medical research, pharmaceutical industries and environmental sciences [38].

Conclusion

With passing years, the burden of wounds is increasing due to rise in treatment cost and antimicrobial resistance. Within available treatment options nanotechnology is found of greater benefits especially metallic nanoparticles being used in wound healing overcome many existing problems. Amidst metallic nanoparticles bi-, tri-, and multimetallic NPs have attracted the greatest interest due to their enhanced catalytic properties and favourable characteristics compared with monometallic NPs. However further studies are essential to assess the effect of bimetallic, trimetallic and multimetallic nanoparticles on wound healing in animal model and to proceed with this formulation development can be done for better activity.

The present review article discussed the effect of various metallic nanoparticles on wound healing and their related studies underwent.

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