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Review Article

Role of Nanoparticles and Nanocomposites in Bone Regeneration

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Bone regeneration is a complex process involving numerous cells like osteoblasts, osteocytes, osteoclasts; various pathways and signalling systems. Usually, human body heals the fractures and degenerations by itself. However, in certain cases like severe trauma, infections, carcinomas the self- repair abilities might get impeded. In such circumstances, bone regenerative materials are used. The commonly used materials include autografts, allografts, isografts, alloplasts etc. The advances in bone regenerative materials comprise of demineralized bone matrix xenograft, phytogenic, synthetic materials. The recent researches focus on polymer materials, tissue-engineered bones, nanoparticles etc. Nanoparticles and nanocomposites are gaining popularity due to its effectiveness in combination with tissue engineering process. Nanoparticles are available in the form of nanotubes, nanocrystals, nanoclusters, nanorods, nanofibers, nanofilms to be used for bone regeneration. Various nanoparticles like gold, silver, zinc, hydroxyapatite, titanium dioxide, calcium copper etc are found to have bone regenerative ability. So, the combination of these bone regenerative nanoparticles with compounds with additive effects could develop nanocomposites with synergistic effects in osteogenesis. Nanocomposites with potential benefits in osteogenesis natural, metal, synthetic and ceramic nanocomposites. This review focus on the potential effects of nanoparticles and nanocomposites on bone tissue and its possibility as a future tissue engineering tool for bone regeneration.

Introduction

Bone is a metabolically active connective tissue that facilitates mobility, protects vital organs, and offers structural support, store house of mineral ions and growth factors, location for haematopoiesis [1]. Basically, bone is composed of cells and the extracellular matrix (ECM), which has both organic and inorganic substances. The balance between bone-forming osteoblasts and bone-resorbing osteoclasts characterizes bone health. characterizes bone health. Bone is a specialized kind of tissue which undergoes continuous remodelling. The necessity for regeneration results due to multiple factors like trauma, infections, carcinomas, inflammatory and regenerative conditions. The regeneration depends primarily on the bone structure, cells and its matrix. Also, bone has the unique ability to regenerate, returning to its pre-injury, fully-functional state [2]. But when the defect is severe, certain times the body will find it difficult to repair itself. In such circumstances, bone regenerative materials (BRM) are utilized.

Autografts, isografts, allografts, xenograft are the commonly used bone grafts [3]. But all these grafts materials have its own demerits [4]. To overcome this and to encourage bone regeneration, new approaches are emerging as a result of advancements in BRMs technologies. Natural or synthetic biomimetic bone materials compatible for regeneration are of widely use. Each year newer interventions and innovative researches by incorporating growth factors, growth stimulants with antibiotics, nanoparticles, nanocomposites are being carried out in developing better regenerative high success rate [5]. But the growth stimulants with antibiotic incorporation also points to one of the global threats of antibiotic resistance or anti-microbial resistance as per the world health organisation [6]. Hence further researches are to be conducted to develop materials without potential threat of antibiotic resistance.

Nanoparticles or nanocomposites with countless advantages have been branded as the material of 21st century [7]. Nanoparticles are

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materials ranging from particle size 1 to 100 nm [8]. Owing to this smaller particle size in opposition to its bulk counterpart is its lower concentration of point defects which leading to its diverse applications. It includes preclinical and clinical medicine, drug delivery systems, nutrient supplements, physics, optics, electronics and so on [9].

Nanoparticles are utilized in different forms in the bone regeneration. Nanostructured scaffolds provide cells with a more supporting framework akin to natural bone structure and regulate cellular proliferation and differentiation, which aids in the regeneration of healthy tissues [10]. Hydroxy apatite, alumina, zinc oxide, titanium, gold, silver, calcium nanoparticles are the common nanomaterials which exhibit bone regeneration abilities or materials which act as scaffold for bone regeneration. Nanoparticles are coupled with drugs, growth factors or biomolecules to help in target drug delivery [11]. This will also aid in osteoimmunomodulation which in turn will promote osteoinduction by creating an environment that is conducive to bone regeneration [12].

A nanocomposite is a multiphase solid substance, in which one phase is having 1 to 3 dimensions less than 100nm. Also, there should be physical and chemical variation among these phases with change in particle size as well. This multiphase aids in superior properties compared to its individual counterparts [13]. These composites are nowadays widely in tissue engineering, drug delivery, medical and dental implants similar to nanoparticles [14].

Commonly used nanocomposites include ceramic based nano composites, metal based nano composites and polymer based nano composites. All of these materials are beneficial in bone regeneration by osteoinduction and osteoconduction [15]. Their tunable properties, in combination with their ability to replicate the natural environment of bone tissue, make them ideal candidates for the treatment of bone fractures, defects and diseases.

The scope of nanoparticles and nanocomposites are limitless owing to its potential to enhance mechanical characteristics of bone scaffolds and thereby increasing the capacity to tolerate physiological loads. It includes their excellent biocompatibility and bioactivity, osteoconductive and osteoinductive properties and antimicrobial properties as well. It also helps in precise visualization of bone morphology and regeneration with the help of nanoparticle-based contrast agents [16].

Hence further researches are crucial in these areas to develop materials with maximum efficiency and least or nil complications and also for incorporating these advances into clinically relevant treatment modalities to improve the patient outcome. Comprehensive characterization and testing of nanoparticle formulations are necessary to mitigate potential risks and maximize their therapeutic efficacy and safety profiles.

Biology of bone

Bone structure

Bones are primarily structured of dense connective tissue called osseous tissue. Osseous tissue basically consists of cells collagen fibres, mineral contents like calcium and phosphate. Essentially, there are two forms of bone: the compact (cortical) bone which makes upto 80% of total bone in the body. It is resistant to bending, torsion and compression. Commonly seen in shaft of long bones like femur, tibia. The second type is the cancellous (trabecular or spongy) bone which makes up to 20% of total bone. Seen in vertebral body, pelvis etc. [1].

Bone cells

Bone constitutes of mainly 30% of organic and 70% of inorganic matrix [17]. Type I collagen makes up the majority of the organic matrix, whereas the non-collagenous component is made up of proteins, lipids, proteoglycans, osteopontin, and proteins found in the bone matrix, such as fibronectin. Nearly 10% of the extracellular matrix (ECM) is made up of non-collagenous proteins (NCPs), which are crucial for tissue metabolism, cell signalling, mineralization of bone, and hierarchical structure [18].

Mainly there are three cell types namely Osteoblasts, osteoclasts and osteocytes [19]. Osteoblasts are primarily responsible for bone formation and the repair of existing bone. They actively synthesize and secrete a protein mixture known as osteoid, which later undergoes mineralization to become mature bone tissue. Additionally, osteoblasts play a role in hormone production, including the synthesis of prostaglandins. Their multifunctional role contributes to the growth, maintenance, and remodelling of bone tissue [20]. Osteocytes are mature bone cells that have become embedded within the bone matrix. They are originated from osteoblasts, which are accountable for new bone formation. Once osteoblasts have completed the process of secreting new bone matrix, they become osteocytes [21]. Osteoclasts are large, multi-nucleated cells responsible for bone resorption. They secrete enzymes and acids that dissolve minerals in bone, allowing for the breakdown and removal of old or damaged bone tissue. Osteoclasts play a crucial role in bone remodelling and can create pathways for nerves and blood vessels to traverse through bone [22].

Bone repair and regeneration

Bone fracture or injury healing is a complex process involving multiple cells like progenitor, inflammatory, endothelial and hematopoietic cells. Also, it involves three phases; inflammation, bone production and bone remodelling [17]. Moreover a four-stage model of bone repair were also developed based on histological observations of fracture healing in both human patients and animal models. It includes Stage 1: Inflammation, Stage 2: Soft callus (Fibrocartilage) formation, Stage 3: Hard callus formation, Stage 4: Bone remodelling [23].

The terminal stage of bone healing is characterised by bone remodelling. The process extends beyond a few months. During this process, the bone regenerates and converts condensed matter, returning to its original shape. Additionally, the blood circulation in the area increases. In contrast to other tissues bone healing does not occur with scar formation. Also, the newly formed bone is eventually indistinguishable from the uninjured bone next to it. [24] However, there are some cases of fracture healing where bone regeneration is impaired with delayed or non-union of the tibia, which can lead to up to 13% fracture healing. In addition to the defective fracture, there are some other scenarios which necessitates bone regeneration like surgical reconstruction of large traumatic and resection defects or in the cases like avascular necrosis or osteoporosis [25].

Current clinical methods for the treatment of bone defects involve natural healing and bridging, grafting, synthetic bone substitutes etc. The conventional standard for the restoration of defective bone healing is by grafting using autografts or allografts. In many cases, bone defects can be healed and bridged by the natural mechanisms of bone repair. This involves ensuring proper alignment and stable fixation of the fractured bone, allowing the body's natural healing processes to take place [26]. Large bone defects can be bridged by callus formation and woven bone. This approach is suitable for bone segments that allow rigid fixation and have adequate vascular

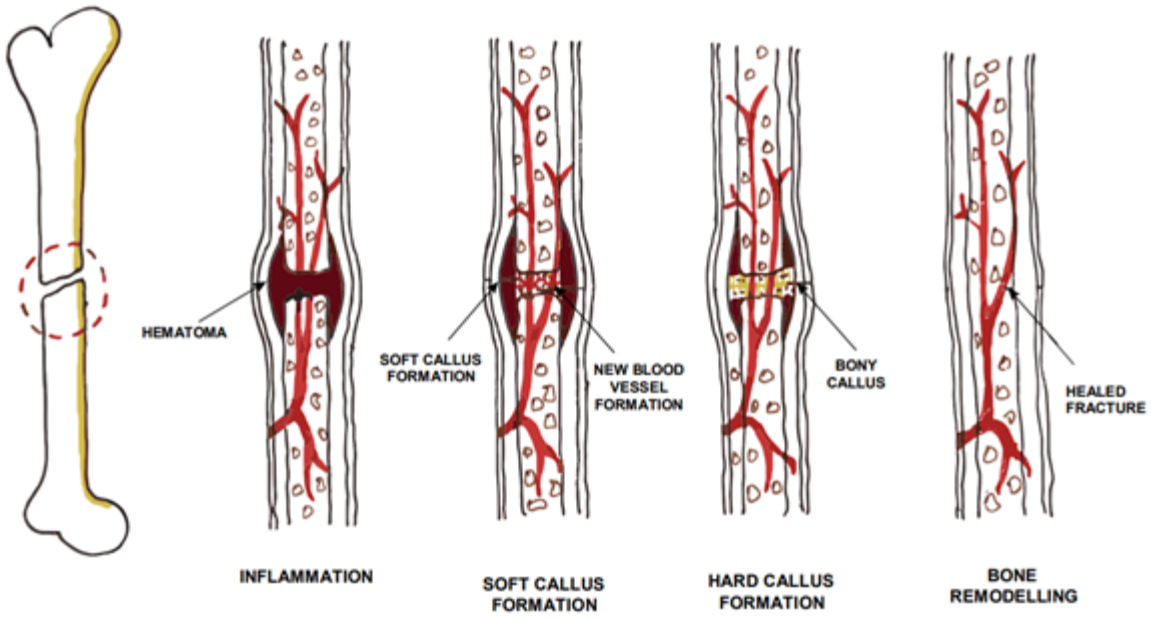

Figure 1: Schematic representation of bone fracture healing

supply [27].

Recent advances in bone regeneration

A wide variety of materials has been experimented to enhance bone regeneration like demineralized bone matrix xenograft, viscous spongy cellulose, bacteria strains secretion-derived bacterial cellulose, phytogenic materials (Gusuibu, coral-based bone substitutes, and marine algae) etc. Similarly, experimentation on synthetic media has also been [28,29]. The recent researches focus on polymer materials, tissue-engineered bones, nanoparticles etc. [30].

Numerous researchers have evaluated a wide variety of materials to find out their role in bone regeneration. Zhang et al used poly l-lactide (PLLA) films to repair traumatic bone defect in rabbits. The cortical bone formation was confirmed with histological findings. They also observed that the poly(L-lactide) inhibited the growth of fibroblasts [31] Diba et al evaluated magnesium-containing bioactive polycrystalline silicate-based ceramics and glass-ceramics. A greater mineral apposition rate was seen in the bone that was newly formed noted in akermanite scaffolds, which improved bone regeneration [32]. Cheah et al suggest that currently, calcium compounds like calcium phosphate, calcium sulfate, tricalcium phosphate and hydroxy apatite offer the greatest synthetic alternative for bone regeneration. The â - tricalcium phosphate and calcium sulphate combination does not requires a membrane for directed bone regrowth [33]. Ielo et al observed that due to the chemical similarity of hydroxy apatite to the inorganic component of bone, it can be employed as a bone grafting material. However pure hydroxy apatite is poorly osteoconductive and extremely delicate. So, improving its crystallinity, surface characters, size, porosity or its combination with other metal ions such as strontium, zinc, magnesium will enhance the osteoconductive potential [34]. Esposito Corcione et al 3D printed composite material based on hydroxy apatite polymers to create personalized scaffolds for tissue engineering applications. They concluded that when compared to pure poly lactic acid, in HA based composite there was a modest rise in the flexural modulus [35]. Fang et al developed a hydroxy apatite mineralized polyacrylamide/dextran hydrogel and observed that this hydrogel based on HA when evaluated in vitro promoted osteoblast adhesion, proliferation, and osteogenic differentiation [36]. Pakulska et al observed that the constituents of photo cross linkable polyanhydrides showed the convincing benefits for orthopaedic regeneration.[37] Helminen et al employed poly—caprolactone-co-lactide as an alternative filler material as a bone deficiency filler material [38]. Matassi et al mentioned that tissue engineering frequently uses biodegradable synthetic polymers as scaffolds, including polyanhydrides, polypropylene fumarate, polycaprolactones, polyphosphazenes, polylactide, polyglycolide, and associated copolymers (polylactide-co-glycolide) [39]. Gao et al evaluated the ability of cell-secreted decellularized extracellular matrixes to promote osteogenic differentiation in reseeded bone marrow mesenchymal stem cells (BMSCs) and observed it to be favourable in ectopic osteogenesis. Furthermore, they suggested the potential utility of decellularized extracellular matrixes in bone tissue engineering [40].

Pountos et al conducted a systematic review regarding the significance of peptides in the healing and regeneration of bone and concluded that peptides increased the bone healing response in experimental setting and hence it gives the possibility of using those for clinical trials [41]. Tavafoghi et al reported that amino acids play a vital role in controlling the mineralization of bone [42]. Bafna et al reviewed Cissus quadrangularis L and concluded its anti-osteoporotic activity. Phytosterols in this species affect osteoblast differentiation and proliferation, which is responsible for the anti-osteoporotic activity [43]. A similar component of plant origin which help in bone regeneration is fucosterol [44]. Oxysterol a component that is derived from the oxidation of cholesterol molecule is found to be bone regenerative. In a study conducted with Oxy49 a cognate of oxysterol found to regenerate bone in a cranial defect in rabbit experimental model [45] Setchell K. D and Lydeking-Olsen E conducted a review on dietary phytoestrogens and their effect on

bone. They reported that phytoestrogens Daidzein and genistein have been found to have a stimulatory effect on protein synthesis and on alkaline phosphatase release by various types of osteoblast cells *in vitro* [46].

Montagnani A et al reported the positive correlation between statins and bone mineral density. BMD was increased in patients supplemented with simvastatin [47]. Bassi et al evaluated bone regenerative ability of microbial biopolymers bacterial cellulose membrane and concluded that in bacterial cellulose membrane group in the rat model, the immunohistochemical analysis of calvarial defect showed the presence of bone biomarkers osteocalcin and osteopontin [48]. Xu et al reported the long-term bone repair ability of tricalcium phosphate (TCP) due to its high alkaline nature and absorbability [49]. Numerous other areas have also been researched, and one area of focus among them is the nanoparticles. A wide range of nanoparticles has reported to have bone regeneration ability.

Biomedical application of nanoparticles

The use of nanoparticles in many biomedical applications, has been gaining interest during the past few years. Nanoparticles has better properties compared to its larger counterpart owing to its surface to volume ratio. Their applications include targeted delivery of drugs, hyperthermia, photoablation treatment, bioimaging, biosensors etc. [50]. Biomedical applications are broadly categorized into two groups: therapeutics (treating diseases, trauma, or morbidity) and diagnostics [51]. Targeted drug delivery, or TDD, is a modern technique, which entails raising the concentration of the drug just in the specific bodily part of interest such as cells, tissues or organs. Increased solubility due to small particle size, increased bioavailability, biodistribution, accumulation of therapeutics in the targeted area, ability to cross blood brain barrier, enter pulmonary system, absorption through tight junctions of endothelial cells makes nanoparticles suitable for targeted drug delivery [52].

When creating target-specific drug delivery systems, metallic, organic, inorganic, and polymeric nanostructures-such as liposomes, micelles, and dendrimers-are commonly taken into account [53]. Watkins et al reported that the bioavailability of natural products can be increased by nanoparticles both in vitro and *in vivo* [54]. Numerous synthetic polymers, including polyvinyl alcohol, poly-L-lactic acid, polyethylene glycol, and poly (lactic-co-glycolic acid), along with natural polymers like alginate and chitosan, find widespread application in the fabrication of nanoparticles at the nanoscale [55].

In recent times, there has been a surge of interest in utilizing nanomaterials to enhance biomedical detection and imaging. This is primarily due to their distinctive passive, active, and physical targeting properties. Nanoparticles, owing to their diminutive size, demonstrate an augmented ability to permeate and accumulate in tumors, resulting in higher concentrations of contrast agent within the local tumor area [56]. Dye- loaded fluorescent silica nanoparticles (SiNPs) are used for non-invasive fluorescent imaging [57]. Lanthanide-doped upconverting nanoparticles (UCNPs) used as imaging contrast reagents [58].

Iron oxide nanoparticles are used as contrast agents in MRI because of its superparamagnetic nature and exhibit magnetization solely when exposed to a magnetic field [59]. Quantum dot-based nanoparticles are reported to be used in the imaging of tumours [60]. Near-infrared (NIR) absorbing carbon nanotubes can be utilized in the imaging of adipose tissue due to its strong attraction towards apolipoproteins and resistance to macrophage phagocytosis. Consequently, they specifically gather on capillary endothelial cells within adipose tissue, while larger vessels remain unaffected [61].

Hyperthermia therapy is a non-surgical procedure which elevates temperature of the tumour to destroy the cancer cells. [62] Kaur et al suggest the potential possibility of using nanoparticle mediated hyperthermia therapy [63].

Nanoparticles and nanocomposites in bone regeneration

One of the current trends is to combine different disciplines or fields to improve medical efficiency. It includes engineering, digital technology, robotics etc. One among them is TERM which conjugates tissue engineering and regenerative medicine. The goal of tissue engineering and regenerative medicine (TERM) is to combine engineering and biological characteristics to provide useful replacements for diseased and damaged tissues. It would be able to improve, maintain, or even restore tissue function [64].

Another issue in bone healing and regeneration is the potential for contamination and infection especially in open fractures. Join Lambert et al mentioned such a contamination with Staphylococcus *aureus* and its resulting complications like failure of removal of microbes and increase susceptibility to osteomyelitis in the sites containing osteoblasts [65]. But combining bone regeneration with tissue engineering is a cumbersome process because of the compounded structure of bone and its continuous exposure to load and stress. This problem can be resolved with nano technology. Nano technology led to the development of structures that are the same size as those seen in naturally occurring bone. Also, it had abilities to cross the biological (microenvironmental, cellular and systemic) barriers [66].

Nanoparticles

Nanoparticles include nanotubes, nanocrystals, nanoclusters, nanorods, nanofibers, nanofilms etc. Nanoparticles have an impact on various aspects of cell behaviour, including cell signalling, proliferation, viability, and integration [67]. Moreover, they play a crucial role in not just supporting cells, but also in governing the function, proliferation, differentiation, and migration of osteoblasts [68]. Nanoparticles which possess regeneration ability so far researched are gold nanoparticles, silver, zinc oxide, hydroxy apatite, calcium, copper, titanium etc. [69].

Gold nanoparticles

Gold nanoparticles find extensive applications in various fields such as diagnostics, drug delivery, biomedical imaging, and photo-thermal therapy. This is primarily attributed to their unique properties including surface plasmon resonance, fluorescence, and

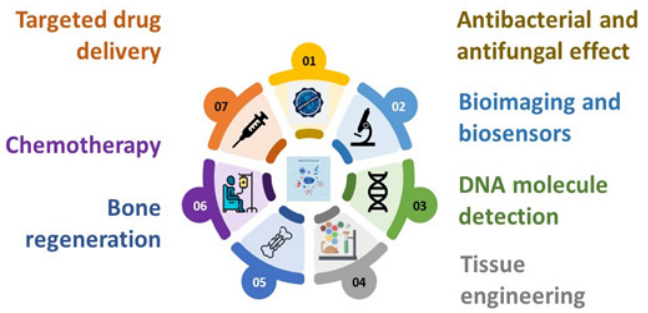

Figure 2: Schematic representation of bio-medical application of nanoparticles

the ease with which their surface can be functionalized. The impact of GNPs on osteogenic differentiation is scientifically evident in vitro Heo et al conducted a study on biodegradable hydrogel loaded with GNPs and reported that the outcomes of the in vitro experiments indicated that the hydrogels containing GNPs enhance the proliferation, differentiation, and alkaline phosphate (ALP) activities of human adipose-derived stem cells (ADSCs) as they transition into osteoblast cells in a manner that depends on the dosage [70]. Gold nanoparticles (NPs) also impact various metabolic processes in the body, thereby directly or indirectly controlling the mechanism of bone regeneration. It also affects scaffold modulation to cellular stimulation [71]. Studies reveal that the gold nano nanoparticles have the potential to enhance the re-accumulation of osteogenic density in defect site. It could also result in amplification of osteogenic genes by the activation of p38 MAPK signalling pathway. Activation of this pathway will also result in differentiation and mineralization of primary osteoblasts [72].

Prevention of bone resorption is also reported as one of the benefits of gold nanoparticles in the bone regeneration. They possess antioxidant potential, produce reactive oxygen species on contrary to RANKL and increase RANKL induced glutathione peroxidase-1 and thereby interrupting the osteoclast formation and henceforth bone resorption [70], in an *in vivo* bone regeneration analysis on a composite based on gold nanoparticles and gelatin nanofibers, it was reported that this composite resulted in the formation of neo-bone, osteocyte in lacuna woven bone and angiogenesis at the site of injury [73]. Shi et al proposes the future use of gold nanoparticles in the treatment osteoporosis, arthritis and bone defects [74]. Based on the study report of Kirdaite et al gold nanoparticles exhibited antioxidant properties, histopathological evaluation showcased reduction of synovial angiogenesis and erosion genesis in the cartilage [75].

Silver nanoparticles

Silver nanoparticles (AgNPs) also have a wide variety of biomedical application. Extensive research has been conducted in silver nanoparticles to enhance the differentiation into osteoblasts. (AgNPs) have the ability to enhance the process of osteogenic differentiation in urine-derived stem cells. Moreover, they can be effectively integrated into tissue-engineered scaffolds that utilize urine-derived stem cells as the primary cells [76].

Lee et al evaluated suppressive impact of (AgNPs) on the differentiation of osteoclasts. The study outcome revealed the inhibitory effect of silver nanoparticles on the activation of certain genes associated with the differentiation into osteoclasts, including c-Fos, NFATc1, RAP, and CTSK, was observed. [77] Literatures also report the osteoinductive properties of (AgNPs). Its ability to regulate the proliferation and differentiation of mesenchymal stem cells involved in bone regeneration. [78] Zhang et al conducted a study to evaluate the ability of AgNPs in the osteogenesis of stem cells. The author transplanted AgNPs to defect area in animal model and observed fracture callus produce, which is suggestive of the osteogenic effect of the nanoparticle and its potential to be used clinically for fracture healing [79].

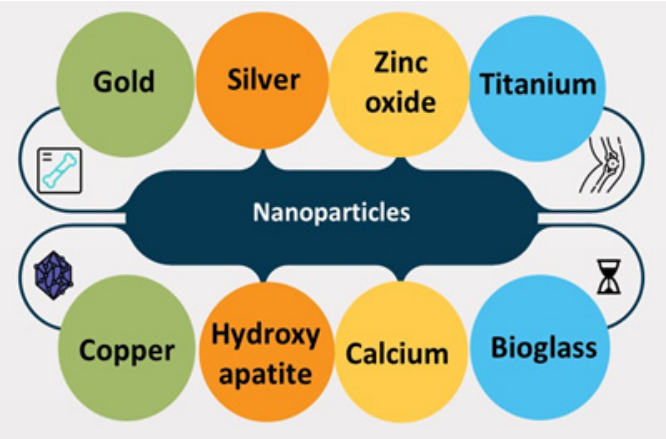

Figure 3: Schematic representation of nanoparticles with favourable bone regeneration ability

AgNPs based natural latex occlusive membrane placed in a calvarial defect in a rat model is reported to produce faster phase of bone regeneration compared to the group without the nanoparticles. Suggestive evidence for the bone regenerative ability of silver nanoparticles [80]. AgNPs were combined with Calcium carbonate nanoparticles and evaluated for bone healing effect. Osteoinductive and osteogenic properties were improved when AgNPs were combined with calcium carbonate nanoparticles than when calcium carbonate nanoparticles used alone. Also, the absorption of calcium carbonate nanoparticles was improved with the combination [81].

AgNPs can be possibly used for fracture healing. This property was exhibited by gelatin-based silver nanoparticle hydrogels. Higher amount of mineralization was demonstrated by the AgNPs hydrogels [82]. Silver nanoparticles are found to be useful in bone surgery instruments as well. This is possible owing to its antimicrobial property. AgNPs can be used to coat the implant surface, bone grafts and membranes, scaffolds for bone. This will prevent microbial contamination and henceforth periimplantitis, which in turn is beneficial for a better osseointegration [83].

Zinc-oxide nanoparticles

Zinc-oxide nanoparticles (ZnO NPs) has extensively researched in different fields including biomedicine is form of zinc containing metal oxide nanoparticles [84]. The human body retains most of its zinc mineral concentration in the bones similar to calcium. [85] Deficiency of zinc will affect the functioning of bone [86]. Zinc also plays a key role in growth and development of bone [87]. ZnO-NPs exhibit osteogenic characteristics via promoting bone development and mineralization. Zalama et al reported the ability of ZnO-NPs to heal bone defect in an animal model when combined with protein rich fibrin [88].

ZnO-NPs also have the advantage of its inexpensive and simple preparation. There are various methods employed for the preparation of ZnO-NPs. Those include physical, chemical, biological procedures. Physical methods are grinding, milling, thermal evaporation, pulsed laser deposition etc. [89]. Chemical methods include photochemical and chemical reduction, sol-gel, chemical precipitation etc. Green synthesis is the biological process for the synthesis of ZnO-NPs. Green synthesis is found to be more beneficial owing to its non-toxic and nature friendly properties. [90] Kalpana et al green synthesized ZnO-NPs using *Lagenaria siceraria* extract are reported its potential to be used for biomedical applications [91]. Bhuyan et al utilized *Azadirachta indica* (neem) leaf extract for the green synthesis of ZnO-NPs. [92] The leaves of *Azadirachta indica* are typically utilized to synthesize ZnO-NPs [93].

ZnO-NPs synthesized based on *S.baicalensis* revealed proliferation of human osteoblast-like MG-63 cells which is suggestive of its role in bone remodelling. Collagen-1 and Alkaline phosphatase expression was observed, which is an early indication of osteogenic

cell differentiation [94]. Prado-prone et al combined ZnO-NPs with polycaprolactone- gelatin membrane and evaluated for its osteogenic potential and observed the presence of calcium and bio-markers of osteoblast. Also reported that this combination also has the ability to decrease the likelihood of infection and encourage the transformation of stem cells already present in the body into osteoblasts, which aids in the regrowth of bone in medical treatments. The combination demonstrated no cytotoxicity as the bone cell viability and proliferation exceeds 70% [95].

One of the mechanisms by which the ZnO-NPs influence the bone formation is by converting MC3T3-E1pre- osteoblastic cells by them into osteoblastic cells and thereby enhancing the alkaline phosphatase activity, synthesis of collagen and osteocalcin. This is an indication of osteoblast differentiation of bone growth. It was also observed that ZnO-NPs would also minimize osteoblast damage caused by mitochondrial malfunction. [96] Fouroutan et al mention the differentiation of human mesenchymal stem cells to osteoblasts when exposed to ZnO-NPs. There was significant expression of genes like osteocalcin, osteopontin, alkaline phosphate in RT-PCR analysis when human mesenchymal stem cells combined with 30 mg mL-1 ZnO-NPs [97].

One of the concerns with ZnO-NPs was its cytotoxicity effect. The primary reason for the cytotoxicity of ZnO-NPs is the significant production of reactive oxygen species (ROS) and the presence of dissolved Zn^{2+} in the culture medium or within cells. [98] [99] However the cytotoxicity is dose-dependent and needs further scientific researches for an evidenced conclusion [100,101].

Hydroxy apatite nanoparticles

Hydroxy apatite (HA) nanoparticles are widely recognized as the building blocks of calcified tissues like bone and teeth [102]. Nano hydroxy apatite scaffolds found to promote the growth and differentiation of human mesenchymal stem cells [103]. Researches with HA nanoparticles report it to be a material which enhances bioactivity of implant and reconstruction bone materials. It also has excellent biocompatibility and osseointegration capabilities [104].

Xu et al compared the effect on bone repair as scaffolds with HA and TCP in combination with poly (lactic-co-glycolic acid) (PLGA) and reported that the HAp /PLGA scaffold outperformed the TCP/PLGA scaffold in terms of mechanical strength and cell proliferation, leading to greater early bone regeneration performance [105]. One of the factors which make nano HA particles favourable material for bone remodelling is its porous nature. Porosity promotes vascularization and angiogenesis [106]. However just porosity is not sufficient for complete bone formation, there has to be pore interconnectivity. Pore interconnectivity will aid in cell and protein adhesion, migration and its differentiation. It also influences fluid penetration and signalling during bone development [107]. Using foam-gel technique Tamai et al developed a completely interconnected porous calcium hydroxyapatite ceramic. They observed new bone formation within a span of 6 weeks with increased strength for the HA material which makes it a superior bone substitute [108].

Numerous researches have been conducted to overcome the disadvantages of HA nanoparticles by combining them with different cells, growth factors, certain natural and synthetic materials. HA and collagen combination is found to have improved properties. Collagen mitigating the low strength or brittle nature of HA [109]. Coupling of HA nanoparticles was found to be useful in bone forming procedure as it improves biocompatibility [110]. Krishnan et al combined Vancomycin with HA nanoparticles and reported that this combination stimulates new bone formation [111]. Osteogenic activity was observed when nano HA particles were co-produced using γ-polyglutamic acid and copper [112].

The mechanism by the nano HA particles enhances the bone tissue regeneration is by promoting cell adhesion, proliferation and differentiation through intercellular contact enhancement. The signalling pathway for HA nanoparticles is through the FGF receptor and Phosphate Transporter, which in turn activate the extracellular signal regulated kinase (ERK) pathway. ERK pathway activates OPN expression which is a bio marker of bone regeneration. The overall advantages of HA nanoparticles include its ability to improve connectivity with the surrounding network. Due to its increased surface area, it absorbs faster, has more molecules on the surface and attach bone cells more compared to conventional hydroxyapatite. Pertaining to its strong bio affinity, it could promote bone integration, collagen I expression, and osteoblast differentiation [113].

Nanocomposites

A nano composite is a material composed of two or more distinct phases at the nanoscale, typically consisting of a matrix phase and a reinforcement phase. The reinforcement phase usually takes the form of nanoparticles, nanofibers, or nanosheets, dispersed within the matrix phase. Nano composites can be designed to enhance biological activity. Human bone itself is a nanocomposite of hydroxy apatite and collagen fibres. Nanocomposites can offer greater benefits than a particular nano material in terms with bone regeneration as a single material cannot reciprocate the properties of bone.

It contributes to enhanced mechanical properties, improved bioactivity, controlled drug delivery, anti-bacterial properties, better biocompatibility, precision and control, promotion of angiogenesis etc to bone tissue engineering. Nano composite materials used for bone regeneration typically involve a combination of biocompatible polymers and nano-sized reinforcements such as nanoparticles or nanofibers. They are employed as polymer-ceramic nano-composites, nano hydrogels, nano fibre-reinforced scaffolds, nano particle-functionalized scaffolds, nano composite coatings, nano films, nano rods etc. Common types of nano composite materials employed in bone tissue engineering include natural, metal-based, polymer-based and ceramic- based nanocomposites.

Natural nanocomposites

Natural or bio-nano composites was widely researched due to its ability to emulate the properties of natural bone tissue. Moreover, it these materials have the advantage of its biocompatibility, biodegradability, bio performance etc. These natural materials are key components of the extracellular matrix, helping to preserve its structure. Additionally, they offer facilitate cellular adhesion. On this note various natural combination have been experimented [114].

Nanocomposites were developed from collagen and hydroxy apatite were evaluated for its role in bone regeneration. It was tested effective to be used in bone tissue engineering as osteoblast-like MG63 cells revealed alkaline phosphatase amplification. The study results also reported its potential to be used as an implant coating for osseointegration because the collagen-hydroxyapatite nanocomposite exhibited osteoconductivity when coated over titanium [115]. Azami et al created a gelatin- hydroxy apatite nanostructured scaffold and assessed its potential for bone tissue engineering. They observed excellent cell attachment, migration, and penetration in the scaffold with improved mechanical strength [116]. Silk fibroin nanocomposites has been reported in the

Figure 4: Schematic representation of nanocomposites with favourable bone regeneration ability

differentiation of bone marrow mesenchymal stem cells [117].

A polysaccharide chitosan based hydroxy apatite nanocomposite were reported for greater levels of MC3T3-E1 cell proliferation. In addition to this greater osteoblastic activity and compressive strength was observed [118]. Hydroxyapatite based nanocomposites are reported to enhance the bone bio marker activities. It has influence on mesenchymal stem cells. The bio markers found to be affected by HA nanoparticles are Runx2, BMP2, Osteopontin, Osteocalcin etc. [119]. Additionally, the absorption of a ceramic nanohydroxyapatite composites is gradual, and after being implanted in the body, HA has the potential to become permanently incorporated into the newly formed bone tissue.

Metal nanocomposites

Various metal-based nanoparticles have the capacity to regenerate bone. Hence various combinations were experimented and nano composites based on metals were created and evaluated for their effectiveness in promoting bone tissue regeneration. Nano composites which shown positive outcomes were gold, silver, titanium etc. These metal nano particles were integrated into hydrogels to create scaffolds for bone regeneration [120].

A multifunctional hydrogel nanocomposite was developed by González-Sánchez et al which exhibited osteoconduction and anti-bacterial effect. The authors propose the potential use of silver nanoparticle-based hydrogel for the purpose of bone regeneration. Also, the cytotoxicity evaluated reported the nano composite to be non-toxic [121]. Xu et al synthesized silver nanoparticle and polyethylene hydrogel from mussel- inspired polydopamine. Real time PCR analysis of the nano-composite revealed the expression of Runx2, alkaline phosphate, bone sialo protein and osteocalcin genes in MC3T3-E1 cells. Also, in-vivo analysis revealed increased healing of the bone defect. These results suggest its potential to be used for bone tissue engineering [122].

Tentor et al created chitosan/ pectin and gold nanoparticle-based hydrogel. This nanocomposite was used as a scaffold. They observed MC3T3-E1 cell growth and division. They also commented on the biocompatibility of the composite [123]. Numerous literatures establish the role of gold nanoparticle- based composite in the osteogenic differentiation of MC3T3-E1 osteoblast-like cells and thereby establishing its role in bone regeneration [124,125] Injectable hydrogels with N-acetyl cysteine attached gold nanoparticles expressed osteogenic activity. Furthermore, they demonstrated favourable biocompatibility and improved mechanical properties [126]. Gold and silver nanoparticle mediated silk fibroin/ nanohydroxyapatite hydrogel composite revealed adhesion of osteoblasts cells [127].

Titanium is considered as gold standard of implants of dental and orthopaedic purposes because of its similar modulus of elasticity as that of bone. Nano application comes to the titanium nanotubes and nanocomposites. Gusmao et al prepared a nanocomposite based on nanohydroxyapatite and titanium nanotubes. Different concentrations of nanocomposite were prepared based on 1%, 2%,3% and 10% weight of titanium nanotubes. The authors reported the increased bone regeneration by the composite in which 10% concentration exhibited the greater bone regeneration [128]. Nano TiO_2 composites of collagen and chitosan was found to be an effective scaffold for the formation of new bone [129].

Polymer nanocomposites

In order to overcome the mechanical strength issue with natural polymers, many synthetic polymers have been evaluated. One among such material is graphene oxide (GO). Due to low toxicity and osteoconductive potential, GO-based composites was introduced foe bone tissue engineering. [130] Al-Arjan et al developed a polymeric nanocomposite using arabinoxylan, apple pectin, hydroxyapatite nanoparticles and GO. They observed bone – scaffold regeneration and suggested the future use of this nanocomposite for fracture healing [131]. Early synthetic polymer employed for guided bone regeneration documented was extended form of Polytetrafluoroethylene (e-PTFE). However, there is potential for microbial infection when e-PTFE exposed to oral environment leading to poor osseointegration and bone tissue regeneration. So, the experimentation replacing e-PTFE was carried out with PGA, PLA, PCL, their co-polymers etc. [132] Poly lactic acid nanocomposites have expressed more bio activeness which makes it suitable as a scaffold for bone regeneration [133].

Zhang et al evaluated elastic properties poly glycerol sebacate nanocomposite and observed that the cell adhesion was poor. [134] Carbon nanotubes have been experimented for bone tissue regeneration. A promotion in osteoblastic function was observed in a nanocomposite based on Carbon nanotubes and polylactic acid [135].

Ceramic nanocomposites

Ceramic based nanocomposites have also been experimented for bone regeneration. Calcium sulfate nanocomposite is found to have osteogenesis potential, in addition to its biocompatibility and bioresorbability. It was reported to increase surface area, which in turn increases the mechanical strength and thereby enhancing osteoconductivity [136]. Lobo et al reported the bone regeneration ability of biphasic calcium phosphate (BCP) ceramics. BCP was reported to be a favourable scaffold for bone formation. The resorption rate of BCP is more compared to hydroxy apatite. When the resorption rate is minimal, there will be apatite layer formation due to release of calcium. This is responsible for the osteoconductive and osteoinductive nature of ceramic materials. Osteoconduction by the material is characterized by the growth of mature osteoblasts cells and direct juxtaposition of bone on to the surface. However, in osteoinduction, acquisition of undifferentiated cells will take place and conversion of the cells to osteoblasts which further result in bone formation [137]. Ramay et al suggested the role of â-TCP and hydroxy apatite nanocomposite in scaffold formation for stress bearing bone reconstruction [138]. BCP, when incorporated with nanocrystals, it exhibited excellent bone regeneration [139].

Multilayered scaffolds were prepared based on Si -substituted nano calcium phosphates. Researchers found that scaffolds made of

silicon promoted a structural transition from nano-HA to nano-TCP, which in turn improved osteoblast adhesion, spreading, growth, and proliferation. These scaffolds could be useful in bone tissue engineering for purposes like regeneration and reconstruction [140]. Literatures have also reported the use of bio glass nanocomposite for bone regeneration purposes. Kim et al developed a bioactive glass and collagen matrix-based nanocomposite and concluded that this nanocomposite induced the formation of bone – like apatite minerals which is suggestive of new bone formation [141]. Bio glass nanocomposites reported to have osteoconductive, osteogenic, antibacterial and angiogenic properties, which could be promising for the development of a newer material for bone regeneration [142].

In general, nano composites show significant potential in improving treatments for bone regeneration by tackling important hurdles like mechanical resilience, bioactivity, drug dispersal, and compatibility with living tissues. Further exploration and advancement in this area are likely to result in the creation of sophisticated nano composite scaffolds, enhancing the effectiveness of bone tissue engineering endeavours.

Conclusion

Bone regeneration is a crucial stage in healing of bony defects. However, in certain conditions, bone regeneration requirement is in excess than the normal potential for self-healing, such as for the reconstruction of large bone defects caused by trauma, infection and tumour resection, as well as skeletal abnormalities, or when the regenerative process is impaired by avascular necrosis or osteoporosis. This necessitates the use of greater potent bone regenerative materials. One of such material is nanoparticles.

Nanoparticles have unique properties like increased surface area, mechanical properties, bioactivity make it's a material of choice for bone regeneration. Its role in bone tissue engineering extends from angiogenesis to osteogenic cell adhesion, differentiation and proliferation. Hence the use of nanoparticles and nanocomposites have significant promising advance in the field of bone regeneration. However, the complete information regarding its biocompatibility, long-term effects, feasibility in production is not yet available. The ongoing and continued researches for its preclinical and clinical outcomes will be crucial and studies could help to fully unlock the therapeutic and applicative capacity of nanotechnology in bone tissue engineering and regenerative medicine.

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