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REVIEW ARTICLE

THERAPEUTIC APPLICATIONS OF NANOTECHNOLOGY IN BONE DISEASES AND BONE TISSUE ENGINEERING

***Mourouguessine Vimal**

Department of Pathology, Sri Manakula Vinayagar Medical College and Hospital, Kalitheerthalkuppam, Madagadipet, Puducherry - 605 107, India

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ABSTRACT

Nanotechnology is an emerging field of research that provides scientists, the opportunity to manipulate matter at atomic and molecular scale. In the field of nanomedicine, nanocarriers are being engineered for targeted drug delivery so as to increase the efficacy and reduce the systemic side effects of the administered drug. This has been fruitful for various chemotherapeutic drugs. Their use in bone diseases is still at its infancy and there is still a long way to go. They are being evaluated as drug carriers for treatment of various bone diseases like tumours, osteoarthritis and osteomyelitis. Nanocarriers exploit the phenomenon of passive targeting through enhanced permeability and retention effect and active targeting by bisphosphonates to preferentially deliver drugs to bone tumours. Also nanoparticles are being tried as scaffolds for bone tissue engineering. This review focuses on the potential of nanotechnology in treating diseases of bone and bone tissue engineering.

Key Words: Nanotechnology, Bone diseases, Bone tissue engineering, Nanoparticles, Nanocarriers.

INTRODUCTION

The history of nanotechnology dates back to 4th century AD when Romans designed the Lycurgus cup which contained gold and silver nanoparticles dispersed in colloidal form in a glass medium. The unique characteristic of this cup is that it appears green when viewed in reflected light and red when light is shone into the cup and gets transmitted through the glass. This is due to the unique properties of the nanoparticles present in trace amounts in the cup. (Freestone *et al.,* 2007) The potential of nanotechnology came to lime light since the famous lecture by the physicist Richard Feynman at California Institute of Technology in 1959. The lecture was titled "There is Plenty of Room at the Bottom", where he explained the possibility of manipulating materials at the atomic or molecular scale for optimising their properties. National Nanotechnology Initiative defines nanotechnology as "*research and development at the atomic, molecular or macromolecular levels in the sub-100 nm range to create structures, devices and systems that have novel functioning properties*". (Morrow *et al.,* 2007)

Priniciple of Nanotechnology

In nanotechnology, a particle is a small object that behaves like a single unit in terms of its transport and properties. Nanoparticles are particles in the size range of 1-100 nm at least in one dimension. (Wilczewska *et al.,* 2012) Nanoparticles can be naturally occuring like viruses, volcanic ash, dust storm and forest fires. They can be produced by man-made processes like welding fumes, diesel exhaust and cigarette smoke. (Buzea *et al.,* 2007)

****Corresponding author: Mourouguessine Vimal,***
Department of Pathology, Sri Manakula Vinayagar Medical College and Hospital, Kalitheerthalkuppam, Madagadipet, Puducherry - 605 107, India.

Also nanoparticles can be artificially created or engineered for various applications. One such is in the field of nanomedicine where nanoparticles are used as carriers of drugs (nanocarriers) in prevention, diagnosis and treatment of diseases. (Rawat *et al.,* 2006) Nanoparticles are being tried as carriers of drugs to the bone cells. They are also being investigated as potential scaffolds for bone tissue regeneration. The advantage of using nanocarriers for delivery of drugs to bone cells is multifold. They are:

1. Systemic side effects are reduced as nanocarriers deliver the drugs only to the target site.
2. Efficacy of the drug increases as more concentration of the drug reaches the target cells.
3. Nanocarriers mask the adverse pharmacokinetic characteristics of the drug. For example, poorly soluble drugs can be converted to water soluble formulations by formulating them as nanocrystals. (Rawat *et al.,* 2006)

Targeting Bone Tissues

Bone tissue can be targeted by two methods, namely passive targeting (targets bone tumour cells) and active targeting.

Passive targeting

It is used for targeting of bone tumour cells. Solid tumours have a rapid proliferation rate and in order to meet their nutritional requirements the process of angiogenesis takes place. But these newly formed blood vessels are abnormal. They have highly disorganized architecture. Their endothelium is fenestrated and discontinuous. The blood vessels lack basement membrane and perivascular smooth muscles. The fenestrations in the endothelium allow selective entry of nanoparticles into the tumour cells. Also these tumour cells lack lymphatic drainage.

Thus when administered intravenously, the nanoparticles preferentially enter the leaky vasculature of tumour tissue and get retained there. This phenomenon is called as "Enhanced Permeability and Retention Effect" (EPR). (Kobayashi *et al.,* 2013)

Active targeting

In active targeting, the surface of nanoparticles is tagged with a particular ligand (like antibodies, folate or peptide fragments like RGD i.e, arginine glycine aspartic acid) which binds specifically to its antigen or receptor present on the target cells. The ligand receptor complex is then endocytosed and the drug reaches the interior of the target cells where they exert their action. (Bertrand *et al.,* 2014) Another prerequisite for this approach is that the antigens targeted should be present in high concentrations in the target cells compared to the other cells. Bisphosphonates have high affinity to hydroxyapatite, which is a main constituent of bone and hence can be used for active targeting of nanoparticle(carrying the drug) to bone. (Gu *et al.,* 2014) The nanoparticles conjugated with bisphosphonates are selectively delivered to bone tissues while sparing the other tissues. Bisphosphonates are drugs used for osteoporosis and paget's disease. They are also being used in treatment of secondary metastasis to bone.

Potential of Nanoparticles to Treat various bone Diseases

Various *in vitro* and *in vivo* studies have been carried out all over the world to detect the potential of nanoparticles to treat various bone diseases and in bone tissue enginnering. Some of these studies have been enumerated in this review.

Primary bone tumours

The most common primary tumour of bone is osteosarcoma. Conventional treatment for osteosarcoma includes chemotherapy, radiotherapy and surgery. Chemotherapy is associated with life threatening side effects due to their off-target actions. This can be minimized by the use of nanoparticles as drug delivery devices which preferentially target the tumour cells sparing the normal cells, due to the enhanced permeability and retention effect. The specificity of nanoparticle binding to the target cells can be enhanced by coating the surface of nanoparticles with antibodies against tumour associated molecules which are expressed in high numbers in tumour cells compared to normal cells. In a study done by Federman *et al.* (Federman *et al.,* 2012), liposomes coated with anti- ALCAM antibodies were used to deliver doxorubicin to the osteosarcoma cells.

It was found that osteosarcoma cells overexpressed a cell surface molecule called ALCAM (activated leucocyte cell adhesion molecule) or CD 166 which could be exploited to enhance the uptake of nanoparticles by the osteosarcoma cells. In this *in vitro* study, it was found that anti-ALCAM antibody coated liposomes bound more avidly to the osteosarcoma cell lines and were rapidly internalized when compared to the untargeted liposomes. This provides a new strategy for intracellular delivery of anticancer drugs in osteosarcoma. Another study was done by Susa *et al.* (Susa *et al.,* 2009) in multidrug resistant osteosarcoma cell lines. They used dextran based polymeric nanoparticles to deliver doxorubicin into these cell lines.

It was found that the nanoparticle loaded doxorubicin were able to penetrate the nucleus of these cell lines more efficiently and caused apoptosis compared to free doxorubicin. Thus this study provides a newer approach to tackle multidrug resistant osteosarcoma with the help of nanocarriers.

Metastatic bone disease

Metastatic bone diseases are commonly seen in tumours of prostate, breast and lung. They are a major cause of morbidity and mortality in these patients. They can cause osteolytic or osteoblastic lesions or both. Breast cancers are known to cause both types of lesions, whereas prostrate cancers cause mostly osteoblastic lesions. Osteolytic lesions are seen with lung cancers. Bisphosphonates are groups of drugs used in the prevention and treatment of metastatic bone disease especially the ones with osteolytic lesions. They cause symptomatic relief by reducing the bone pain in these patients. Also the nitrogen containing bisphosphonates have direct anti-tumour activity. They inhibit angiogenesis, adhesion and invasion of tumour cells. (Gu *et al.,* 2014) Bisphosphonates bind to the bone matrix and get released when osteolysis occurs by the action of osteoclasts. The released bisphosphonates are taken up by the osteoclasts and induce cell death. Also bisphosphonates can be used for active targeting of nanoparticle-drug conjugate to bone.

In a study done by Clementi *et al.* (Clementi *et al.,* 2011), polyethylene glycol (PEG) dendrimer conjugated with alendronate (an aminobisphosphonate) was used to deliver paclitaxel into tumour cells. This conjugate produces active targeting by alendronate and passive targeting through enhanced permeability and retention effect. The conjugate produced a greater binding to hydroxyapatite *in vitro* and was more cytotoxic to the tumour cells compared to the free drug. Toxicity of paclitaxel is due to the drug itself and its solubilizing formulation cremophor EL. Paclitaxel-PEG-alendronate conjugate was soluble in physiological solutions, thus eliminating the need for Cremophor EL. Another study done by Chaudari *et al.* (Chaudhari *et al.,* 2012) showed that zoledronate conjugated PLGA (Poly lactic co-glycolic acid) nanoparticles carrying docetaxel showed more uptake in tumour cells compared to pegylated PLGA nanoparticles. In animal studies, technetium-99 radiolabelled zoledronate tagged nanoparticles exhibited prolonged blood circulation half life, reduced liver uptake and enhanced retention at the site of bone tumour. However, further animal and human studies are needed to prove that bisphosphonate conjugated nanoparticles carrying the chemotherapeutic agent are more efficacious and safer compared to the free drug.

Osteoarthritis

Osteoarthritis is a chronic debilitating condition in which there is degeneration of the articular cartilage and subchondral bone. Treatment modalities include lifestyle modifications, analgesics, intra-articular injection of drugs like corticosteroids and the last resort is joint replacement. The role of nanoparticles in increasing the retention time of osteoarthritis drugs injected into the joints is being explored. In a study by Morgen *et al.* (Morgen *et al.,* 2013), the cationic polymeric nanoparticles were found to interact with the hyaluronate present in the synovial cavity and form hydrogel. This increased the retention time of these drugs in the joint space.

The cationic polymeric nanoparticles were synthesized from dextran which contained a neutral polymer core and cationic surface polymer. Small fluorescent peptides (drug mimics) were conjugated via ester bond to the polymer. When these nanoparticles were mixed with human osteoarthritis synovial fluid, cross linked hydrogels were formed which were visualized by fluorescence microscopy. Also intra articular injection of these nanoparticles into the rat knee joint showed increased local retention compared to the fluorescent labelled free tetrapeptide. Thus this provides a new approach for achieving increased retention and sustained delivery of drugs into knee joint for treatment of osteoarthritis.

Osteomyelitis

Osteomyelitis refers to infection of the bone. Osteomyelitis is traditionally treated with systemic antibiotics and surgical debridement of necrotic bone. This conventional treatment has several disadvantages like increased side effects due to repeated systemic antibiotic therapy and bone loss due to surgical debridement of necrotic bone. These problems can be overcome by using nanoparticles as drug carrier systems for targeted antibiotic therapy. Calcium phosphate nanoparticles are tried as antibiotic delivery platforms in osteomyelitis. (Desai and Uskoković, 2013) This is because calcium phosphates are natural components of bone. Also they are biocompatible, osteoconducive and could be prepared into nanoforms.These calcium phosphate nanoparticle drug conjugate acts locally (due to targeted therapy) and systemic side effects of antibiotics can be overcome. These particles can be designed to provide local and sustained drug delivery at the site of infected bone. Also the calcium phosphate nanoparticles augment the osteogenic response to osteoblasts, providing osteoconducive environment. Thus there is a drastic reduction in the amount of bone that gets surgically debrided.

Bone tissue Engineering

Conventionally bone grafts were used to augment bone repair and regeneration in patients with bone defects caused due to trauma, infection, tumour resection or congenital deformity. Autologous grafts or allogenic grafts were used for this purpose. Autologous grafts are considered as gold standard for bone grafts because they posses the property of osteoconduction and osteoinduction, no risk of transmission of infection and low graft rejection rate. (Amini *et al.,* 2012) The drawbacks of autologous grafts are that they lead to additional morbidity due to surgery at donor site. The problems with allogenic graft are that they are difficult to get, risk of transmission of infections, prone for rejections and less osteoinductive property. A novel approach to tackle these bone defects is by bone tissue engineering where we reconstruct bone tissue by using biodegradable and porous scaffolds (resembling extracellular matrix of bone) containing osteogenic cells, pluripotent stem cells and growth factors (like bone morphogenic protein). The main constituents of bone extracellular matrix like type 1 collagen fibrils and hydroxyapatite crystals are nanometers in diameter and hence they interact better with nanoscaled structures. (Harvey *et al.,* 2010) This explains the importance of nanotechnology in the field of bone tissue engineering. Typically there are two approaches to use nanoparticles in designing scaffold for bone tissue engineering.

One is to incorporate nanoparticles carrying drugs or growth hormones into the scaffolds for tissue reconstruction. The other is to construct the scaffold using nanoparticles. Direct incorporation of growth factors or bone morphogenic proteins into the scaffolds by adsorption may lead to uncontrolled burst release on implantation and cause bone hyperplasia. This can be overcome by incorporating nanocarriers carrying growth factors into the saffolds. This leads to controlled and sustained release of growth factors favoring bone regeneration. Nanospheres have been used for this purpose. (Wang *et al.,* 2012)

These nanospheres are made up of biodegradable and biocompatible materials like natural polymers (collagen, gelatin, fibrin, chitosan and alginates) or synthetic polymers (Poly lactic acid, ploy glycolic acid, poly lactic co-glycolic acid and poly caprolactone) and inorganic substances (like calcium phosphate). In a study done by Winkins *et al.* (Winkins *et al.,* 2014) polycaprolactone scaffolds were engineered and incorporated with albumin nanoparticles containing resveratrol. Resveratrol promotes differentiation of bone marrow derived mesenchymal stem cells to osteoblasts, thus aiding in bone repair and regeneration. This study demonstrated a prolonged release of resveratrol from the polycaprolactone scaffold and hence could promote osteogenesis in bone tissue engineering. In the second approach, nanospheres are used as building blocks of the scaffold. This provides various advantages like: (Wang *et al.,* 2012)

- increased porosity of scaffold thus facilitating osteoblast attachment and proliferation.
- increased mechanical strength to scaffold.
- nanoparticles can be embedded with biomolecules leading to their controlled and sustained release.

Instead of nanospheres, nanofibres have also be tried as scaffolding components in bone tissue engineering. (Christenson *et al.,* 2007) Their advantages are high porosity, high surface to volume ratio and morphological similarity to natural extracellular matrix of bone. Various biodegradable and non biodegradable polymers are used for preparing nanofibres. Biodegradable polymers are preferred, as on disintegration they produce spaces which act as potential sites for osteoblast attachment, proliferation and production of extracellular matrix. Thus nanoparticles show lot of potential to be used as scaffolds in bone tissue engineering, however further *in vivo* and clinical studies are needed to prove their efficacy and safety.

Conclusion and Future Prospects

Nanoparticles are being tried in targeted drug delivery to bone cells. Passive targeting and active targeting are the two ways of targeting bone cells. Passive targeting is mainly for targeting bone tumour cells, exploiting the phenomenon of “enhanced permeability and retention effect”. Bisphosphonates can be used for active targeting of the nanoparticle-drug conjugate to bone cells. Several in vitro and in vivo studies have demonstrated the use of nanoparticles as drug carriers in various bone diseases like bone tumours, osteoarthritis and osteomyelitis. Nanoparticle conjugated chemotherapeutic drugs are found to be taken up into the bone tumour cells lines better than the free drug.

Bisphosphonates can be used for active targeting of nanoparticles carrying chemotherapeutic drug in secondary bone metastasis. Also bisphosphonates have antitumour activity. Nanocarriers have also shown to increase the retention time of drugs injected intra-articularly in osteoarthritis models. Calcium phosphate nanoparticles are tried for targeted delivery of antibiotics to bone tissue in osteomyelitis. Nanoparticles are also being incorporated in the scaffolds for bone tissue reconstruction. Also nanoparticles tried as building blocks of the bone scaffolds. A though nanotechnology shows a promising face in treating bone diseases in vitro and in vivo experiments, further studies are needed to prove its efficay and safety in humans. If proven fruitful, it will open a new era of nanotechnology based treatment of bone diseases.

REFERENCES

Amini, A.R., Laurencin, C.T. and Nukavarapu, S.P. 2012. Bone tissue engineering: recent advances and challenges. *Crit. Rev Biomed Eng.,* 40:363-408.

Bertrand, N., Wu, J., Xu, X., Kamaly, N. Farokhzad, O.C. 2014. Cancer Nanotechnology: The impact of passive and active targeting in the era of modern cancer biology. *Adv. Drug Deliv. Rev.,* 66:2-25.

Buzea, C., Pachec,o I.I. and Robbie, K. 2007. Nanomaterials and nanoparticles: sources and toxicity. Biointerphases, 2:17–71.

Chaudhari, R.K., Kumar, A., Khandelwal, M.V.K., Ukawala, M., Manjappa, A.S., Mishra, A.K., *et al*. 2012. Bone metastasis targeting: a novel approach to reach bone using zoledronate anchored PLGA nanoparticle as carrier system loaded with docetaxel. *J. Control Release,* 158:470–8.

Christenson, E.M., Anseth, K.S., van den Beucken, J.J.J.P., Chan, C.K., Ercan, B., Jansen, J.A., *et al*. 2007. Nanobiomaterial applications in orthopedics. *J. Orthop. Res.,* 25:11–22.

Clementi, C., Miller, K., Mero, A., Satchi-Fainaro, R. and Pasut, G. 2011. Dendritic poly(ethylene glycol) bearing paclitaxel and alendronate for targeting bone neoplasms. *Mol. Pharm.,* 8:1063–72.

Desai, T.A. and Uskoković, V. 2013. Calcium phosphate nanoparticles: A future therapeutic platform for the treatment of osteomyelitis? Ther Deliv., 4:643–5.

Federman, N., Chan, J., Nagy, J.O., Landaw, E.M., McCabe, K., Wu, A.M., *et al*. 2012. Enhanced growth inhibition of osteosarcoma by cytotoxic polymerized liposomal nanoparticles targeting the alcam cell surface receptor. Sarcoma. 126906.

Freestone, I., Meeks, N., Sax, M. and Higgitt, C. 2007. The Lycurgus cup - A Roman nanotechnology. *Gold Bulletin,* 40:270-7.

Gu, W., Wu, C., Chen, J. and Xiao, Y. 2013. Nanotechnology in the targeted drug delivery for bone diseases and bone regeneration. *Int. J. Nanomedicine,* 8:2305–17.

Harvey, E.J., Henderson, J.E. and Vengallatore, S.T. 2010. Nanotechnology and bone healing. *J. Orthop. Trauma,* 24:S25–30.

Kobayashi, H., Watanabe, R. and Choyke, P.L. 2013. Improving conventional enhanced permeability and retention (EPR) effects; what is the appropriate target? Theranostics, 4:81–9.

Morgen, M., Tung, D., Boras, B., Miller, W., Malfait, A.M. and Tortorella, M. 2013. Nanoparticles for improved local retention after intra-articular injection into the knee joint. *Pharm. Res.,* 30:257–68.

Morrow, K.J., Bawa, R. and Wei, C. 2007. Recent advances in basic and clinical nanomedicine. *Med Clin. N. Am.* 91:805-43.

Rawat, M., Singh, D., Saraf, S. and Saraf, S. 2006. Nanocarriers: Promising Vehicle for Bioactive Drugs. *Biol. Pharm. Bull.,* 29:1790—8.

Susa, M., Iyer, A.K., Ryu, K., Hornicek, F.J., Mankin, H., Amiji, M.M., *et al*. 2009. Doxorubicin loaded Polymeric Nanoparticulate Delivery System to overcome drug resistance in osteosarcoma. *BMC Cancer,* 9:399.

Wang, H., Leeuwenburgh, S.C.G., Li, Y. and Jansen, J.A. 2012. The use of micro- and nanospheres as functional components for bone tissue regeneration. *Tissue Eng Part B Rev.,* 18:24–39.

Wilczewska, A.Z., Niemirowicz, K., Markiewicz, K.H. and Car, H. 2012. Nanoparticles as drug delivery systems. *Pharmacol. Rep.,* 64:1020–37.

Winkins, S., Kamath M. Dhanasekaran, and Ahmed, S. 2014. Polycaprolactone scaffold engineered for sustained release of resveratrol: therapeutic enhancement in bone tissue engineering. *Int. J. Nanomedicine,* 9:183-95.

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