Nanotechnology towards prevention of anaemia and osteoporosis: from concept to market

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ABSTRACT
The application of nanotechnology in medicine, diagnostics, therapeutics, drug delivery, etc. in order to overcome the limitations faced by these fields has already been established. Anaemia and osteoporosis are two of the most widely prevalent iron and calcium deficiency diseases among women. Conventional drugs for both have their inherent limitations which can be overcome by nanotechnology. Nanonization of drugs has been established as a very efficient way to enhance the bioavailability and absorption of the drug in the gastrointestinal tract. In addition to this, encapsulation of drugs in solid lipid nanoparticles is also employed to increase the stability of the drug in the gastrointestinal tract and to facilitate its controlled release. Nanotechnology is also applied in bone tissue engineering to prepare bone grafts for bone injury. Nanotechnology-based bone grafts have shown to possess enhanced mechanical properties and better biocompatibility and osteoconduction than the conventional bone grafts. This review article highlights the research trends and challenges of nanotechnology based drugs to combat iron deficiency anaemia and osteoporosis. This review outlines the recent research trends in nano-drugs and touches on issues concerning the market, industries and patents pertaining to drugs for anaemia and osteoporosis. To provide the safety and risk factors of nanomaterials, we have briefly highlighted the toxicological aspects of nanomaterials used in drugs.

Introduction
Nanotechnology has emerged as a science that has the potential to revolutionize any field it is applied to. Among the many applications of nanotechnology, one is the targeted delivery of specific nutrients, minerals, molecules or drugs. Recently, there has been a surge in the development of nanotechnology-based delivery systems for the targeted delivery of many drugs or to enhance the bioavailability of certain micronutrients in the body. The primary applications of nanomaterials in drug delivery are: to make the drug safer and more biocompatible, to reduce its toxicity without compromising with its therapeutic potential, for specific and targeted drug delivery and to increase its bioavailability and absorption in the body [1]. Entrapment of the drugs in nanomaterials is done to overcome the limitations presented by the bulk non-encapsulated form of the drug. These limitations might be due to the insolubility of the drug in a particular form or conditions, susceptibility of the drug to physical or chemical damage, enzymatic degradation, damaging effect of the drug on the gastrointestinal tract (in case of oral intake), etc. Nano-size reduction of the drugs greatly enhances and alters their properties like stability, solubility, absorption, bioavailability, mechanism of action, etc. [2–4]. Min et al. [5] reported that the drug stability and tumour-targeting ability of camptothecin, encapsulated in glycol chitosan nanoparticles that have been hydrophically modified by conjugating 5-fluouracil acid moieties, were higher than those of free camptothecin, as observed in mice models. Lee Jia [6] reported that nanonization of two poorly soluble drugs, carbamazepide and thiadiazole derivative, increases their oral bioavailability both in vivo (SCID mice and Sprague–Dawley rats)and in vitro (Caco-2 cell lines) models. When the drugs were reduced to a size of 280 nm by pearl milling, their bioavailability was much higher than that of the micro-sized counterparts [6]. Mahler et al. [7] showed that oral exposure to polystyrene nanoparticles affected iron uptake and absorption through in vivo studies in chickens and in vitro studies in Caco-2 and HT29/MTX cell lines. High doses of nanoparticles caused the disruption of the cell membranes and...
increased the iron transport through them. Similar results were obtained from the intestinal loop model of the chicken, where acute exposure resulted in lower iron absorption than in chronically exposed chickens. Acute oral exposure to nanoparticles disrupted the iron transport, whereas chronic exposure to high doses of nanoparticles increased the iron transport and iron absorption by increasing the surface area of the intestinal lumen available for iron absorption by remodelling the intestinal villi [7]. This study provides valid evidence for the increase in absorption of molecules when the gastrointestinal tract is exposed to nanoparticles and can be exploited to increase the bioavailability of nutrients and drugs that are administered orally.

These properties of nanomaterials can be exploited to create formulations or delivery systems that enhance the bioavailability and absorption of specific nutrients or minerals. They can be used to combat deficiency diseases that are widely prevalent in both developing and underdeveloped countries [8–15]. Anaemia and osteoporosis are two of the most widely prevalent deficiency diseases, particularly among women. In this review, we discuss the recent research trends, challenges and opportunities of nanotechnology-based delivery systems for the benefits of women mainly – to overcome anaemia and osteoporosis.

Nanotechnology for iron deficiency anaemia
Iron deficiency is one of the most common nutritional disorders in the world prevailing in both developed and underdeveloped countries. Adequate iron concentration is crucial in the body for various metabolic processes like oxygen transport and storage, hormone synthesis, DNA replication and repair, cell cycle control and various enzymatic reactions. Iron deficiency anaemia (IDA) is caused by prolonged low levels of iron in the blood. Low levels of iron in the blood may be due to a variety of factors like iron-deficient diet, worm infestations, blood loss due to bleeding, intestinal diseases, etc. Figure 1 gives a list of the factors responsible for iron deficiency anaemia. It is particularly more prevalent in women of child-bearing age and children because of their increased requirement for iron for growth and developmental processes. According to the estimates of the World Health Organization (WHO), 48% of children between 5 and 14 years and 52% of pregnant women in developing countries are anaemic [8]. Figure 2 shows the prevalence of anaemia among women belonging to different regions of the world. Plant-based diets have a high level of non-heme iron, which is poorly absorbed in the body as compared to heme iron, found in animal tissues [9]. This is another reason for iron deficiency in the developing countries which rely mainly on food crops and plant-based diets.

![Figure 1. Factors responsible for iron deficiency anaemia.](image-url)
The presence of absorption inhibitors like phytic acids, phenolic compounds, and calcium in cereals and pulses also results in the low absorption of iron in the blood. Phytic acids and polyphenolic compounds cause chelation of the iron and reduce its absorption into the bloodstream [10].

Conventional strategies to deal with iron deficiency include iron fortification in food crops and iron supplementation. Development of iron biofortified crops is a good option to combat iron deficiency in rural areas which depend mainly on agriculture [11]. However, there is a significant risk of excess iron as such crops will be consumed even by the non-deficient people. High levels of iron in the blood are associated with heart disease, kidney disease and diabetes. Iron supplementation relies on use of iron salts like ferrous sulphates, ferrous citrate, etc. Ferrous salts are mostly used because of their low cost and availability. However, they are highly reactive and are known to cause severe effects on the gastrointestinal tract and also affect the natural microbiota of the digestive system [12]. Moreover, iron is poorly disposed off from the body; long-term consumption of artificial supplements should be avoided to prevent cellular accumulation of iron, which can lead to oxidative stress, lipid peroxidation and cancer. Thus, there is a need for a new form of therapy or a new form of iron supplementation that can improve the bioavailability of iron in the body by increasing its absorption to treat iron deficiency in a shorter duration and quickly enable the body to overcome anaemia. For these reasons, researchers and enterprises are now interested in employing nanotechnology for the successful delivery and enhancement of bioavailability of iron in the body.

Anaemia has attracted considerable attention of food scientists and technologists around the world and many nanostructure-based delivery systems for iron are being developed or research on such structures is being carried out. Only water-soluble forms of iron are suitable for food fortification but they result in undesirable colour changes and off-taste to the food, which rules out their direct use as food fortificants [13]. The physiological forms of iron used for supplementation or fortification are known to have harmful effects on the gastrointestinal tract and also on the natural microbiota of the digestive system. Also, the generation of free radicals causes inflammation of the intestinal mucosa. Encapsulation of iron in a nanostructure ensures that it is delivered safely without having any harsh effects on the gastrointestinal tract. Encapsulation also ensures enhanced bioavailability and absorption because a decrease in the particle size increases its absorption through the gastrointestinal tract. Also, nano-size reduction increases the solubility and absorption of the molecules. This has been validated by the findings of Wu et al. [14], who report that the absorption of non-heme iron is enhanced by anchovy muscle protein hydrolysate (AMPH) by the formation of nano-sized ferric hydrolysis products. They evaluated the effect of AMPH on the bioavailability and intestinal absorption of non-heme iron in rats and also studied the cellular uptake mechanism in vitro using Caco-2 cells. It was observed that the bioavailability was enhanced and the haemoglobin regeneration efficiency was increased [14]. This proves...
that, in the nano-form, the bioavailability of the particles, here Fe, increases in the body.

Several studies have been conducted on how nanostructures can be formulated for the encapsulation of elemental iron or ferrous salts and their successful delivery inside the body to combat iron deficiency. Pereira et al. [15] have developed 5–10 nm particles of iron oxo-hydroxide and modified them by the addition of tartaric and adipic acid tails to make them mimic the ferritin core. Ferritin is an iron storage and detoxification protein found in plants, animals and humans. They used animal and cellular models to test the efficacy and uptake of iron oxo-hydroxide nanomaterial by the cells. The toxicity was also tested on the human intestinal Caco-2 cell line and rats. The nanomaterial was found to have minimal cytotoxic effects on the cells and no undesirable effects were reported on the intestinal mucosa and faecal microbiota of the rats. Such a preparation has a strong potential to be used for the treatment of iron deficiency anaemia, as it does not have any significant toxic effects on the cells and the microflora of the gastrointestinal tract and has good solubility in the gastric acids. Moreover, the ligand modified nano-Fe structure closely resembles the naturally occurring ferritin found in the human body and hence, its uptake into the blood is enhanced. In the studies performed on mildly iron deficient menopausal female subjects, to determine the bioavailability of various ligand modified iron oxo-hydroxide nanoparticles, it was found that the absorption of ligand doped Fe (III) oxo-hydroxide nanoparticle was 80% as efficient as that of ferrous sulphate, commonly used in iron supplementation. The former was also three times better absorbed than the simple ferric chloride salt, doped with the same ligand. In addition, in the rat models, administration of iron oxo-hydroxide nanoparticles showed no structural or physiological changes and no abnormal Fe deposition was reported following 14 days of feeding [15]. Thus, it can be said that such a formulation is safer and more efficient than most available conventional iron salts.

Zariwala et al. [16] prepared solid lipid nanoparticles loaded with ferrous sulphate by the double emulsion solvent evaporation process. Solid lipid nanoparticles are widely used in drug targeting and delivery because of the multi-fold advantages they provide. Solid lipid nanoparticles provide the advantage of high absorption, biocompatibility and stability, unlike liposomes which are often unstable. The authors tested the absorption of the nanomaterial using human intestinal Caco-2 cell lines and used ferritin formation as a marker for iron absorption. Iron absorption from ferrous sulphate loaded lipid nanomaterial was found to be 13.42% higher than that from free ferrous sulphate. The higher absorption can be attributed to the presence of lipids in the nanomaterial. Lipids are known to enhance the intestinal absorption of encapsulated drugs or molecules by promoting particle transport across the bimolecular lipid membrane of the gut [17]. Lipids also exhibit bioadhesive properties and adhere to the lining of the gastrointestinal tract, enhancing the cellular uptake [16,18]. Lipids can also modify the barrier function of the gastrointestinal tract by acting as permeability enhancers [19]. The cytotoxicity of solid lipid nanoparticles was tested in Caco-2 cell lines using the MTT (methylthiazolyldiphenyl-tetrazolium bromide) assay. Solid lipid nanoparticles were found to have no significant cytotoxic effects on the Caco-2 cells. Since lipids are biocompatible with the biological systems, solid lipid nanoparticles can emerge as a very efficient delivery system for iron. It would overcome almost all the limitations and demerits presented by conventional iron supplementation drugs.

Another study on solid lipid particles was done by Hosny et al. [20], who developed solid lipid nanoparticles loaded with iron. The solubility of iron in various solid lipids was measured and the effect of variation of various parameters of synthesis like the type and concentration of surfactant, duration of homogenization and ultrasonication on the particle size, zeta potential and entrapment efficiency was also determined. Iron solid lipid nanoparticles were prepared by hot emulsification and ultrasonication. The drug release was evaluated by the dialysis bag method and an in vivo pharmacokinetic study was done on male albino rabbits that were kept in a fasting state for 24 hours prior to the experiment. The evaluation of iron solid lipid nanoparticles was done in comparison to the iron tablets available in the market. It was found that the drug release efficiency and the bioavailability of iron were higher in solid lipid nanoparticles than in the iron tablets available in the market. The bioavailability of iron was increased fourfold by its incorporation into the solid lipid nanoparticles. Iron salts have poor aqueous solubility and iron formulation into solid lipid nanoparticles enhanced both its solubility and tissue permeability. The presence of lipids also facilitates absorption by various mechanisms like intracellular or paracellular uptake through Peyer’s patches, bypassing the liver by direct absorption into the lymphatic circulation. They can also be administered by various routes such as tropical, oral, ophthalmic, rectal and parenteral routes [20]. Thus, such lipid nanoparticle delivery systems can very efficiently enhance the release and bioavailability of iron in the body. Lipids, however, have a tendency to clog the pores and channels in the cell membranes, which still needs to be thoroughly investigated.

Ferric pyrophosphate is a water insoluble iron compound which is used as a food fortificant in chocolate
drinks and infant cereals to enhance the iron absorption. Due to its low solubility in dilute acids, it dissolves poorly in the gastric acids and, hence, is poorly bioavailable for absorption. Srinivasu et al. [13] synthesized nano-sized ferric pyrophosphate \( (\text{FeO}_4(P_2\text{O}_7)_3) \) by titration of sodium pyrophosphate solution against ferric chloride in the presence of ethylene glycol at 65 °C with continuous stirring to avoid agglomeration of the particles. The bioavailability of nano-sized ferric pyrophosphate was evaluated in iron-depleted rats by feeding them with food fortified with nano-sized ferric pyrophosphate for 15 days. A part of the experimental rats were fed with the same dose of \( \text{FeSO}_4 \) for the same duration to compare the level of bioavailability in both the experimental groups. The relative bioavailability of ferric pyrophosphate was found by calculating the haemoglobin regeneration efficiency. Its relative bioavailability was calculated to be 103.2% with respect to that of ferrous sulphate. Oral toxicity studies in rats gave no indication of toxic effects of nano-sized ferric pyrophosphate. The higher bioavailability of nano-sized ferric pyrophosphate than ferrous sulphate can be attributed to the increased solubility of ferric pyrophosphate in dilute acids because of the reduction in particle size. It is an established fact that the solubility of metals increases with the decrease in particle size [13].

Another nano-based formulation for iron deficiency treatment was attempted by Salah et al. [21]. They developed magnetite \( (\text{Fe}_3\text{O}_4) \) nanoparticles capped with vitamin C (ascorbic acid), since iron absorption in the intestinal lumen is facilitated by vitamin C. The effectiveness of the formulation was tested on albino rats. It was found that intra-peritoneal or oral administration of the above drug can effectively cure iron deficiency anaemia. Bone marrow studies of the experimental rats showed that the magnetite nanoparticles capped with vitamin C stimulated erythropoiesis without affecting the haemostasis. Administration of a single dose increased the haemoglobin concentration from 7 to 14 g/dL and the red blood cell counts increased from \( 3.2 \times 10^6 \) to \( 6.5 \times 10^6/\text{mm}^3 \) within 10 days [21]. European Egyptian Pharmaceutical Industries (EEPI) holds a patent for the development of iron-based nanocomposites for the treatment of iron deficiency anaemia [22]. Iron oxide (magnetite) nanoparticles capped with a mixture of multivitamins, folic acid, nicotinic acid and ascorbic acid was developed. Folic acid and nicotinic acid are important for cell formation and vitamin C is required for the absorption of iron from the intestinal lumen. The authors developed two forms of the drug: gel-based and aqueous solution for oral administration. The formulation was tested in five-week-old albino rats, in which anaemia was induced by withdrawing blood from the canthus of eyes. A healthy, non-anaemic group of animals was kept as the control. The rats were fasted for six hours prior to administration of drugs. The experimental rats were administered different concentrations of nano iron oxide depending on their body weight, ferric chloride (for comparison) or magnetite nanoparticles coated with multivitamin mixture. It was observed that just a single dose of 0.8 mg of magnetite nanoparticles capped with multivitamin mixture was able to treat iron deficiency anaemia and to recover the erythrocytes in less than four days. The haemoglobin levels rose from 4.4 to 14.6 g/dL in 7 days and stabilized at 13.6 g/dL 80 days after administration. This is much faster than the results with ferric chloride. The vitamin mixture was able to correct anaemia but did not reach the same level as magnetite nanoparticles. Thus, a single dose of magnetite nanoparticles capped with multivitamin mixture, equivalent to 8.3 mg, can correct iron deficiency anaemia in albino rat in less than four days. This is faster and more efficient than most commercially available treatments. In addition, because of the low concentration of nano iron oxide required to significantly correct anaemia, the drug has minimal toxic side effects on the body, unlike conventional iron tablets which have a lot of side effects [22]. Along with nano-size magnetite particles, vitamin C also enhances the absorption of Fe through the intestinal villi. Thus, this combination can enhance the bioavailability of iron and has the potential for being commercialized as a treatment of iron deficiency. Such a product has a lot of scope to be used for treatment of iron deficiency but there are certain parameters that still need to be investigated or evaluated, like, the solubility of the nanoparticles in the gastric acids, their effect on the gastrointestinal cells, their biocompatibility and bioavailability in the human body and long-term effects of their consumption. Since the physiology of the human body is different from that of rats, the effects of magnetite nanoparticles need to be tested in human trials as well, prior to their commercial use as a therapeutic agent.

Thus, it is quite clear that the use of nanostructures for the delivery of iron or iron salts provides benefits that are absent in conventional remedies for iron deficiency. The use of nanomaterials has a potential to circumvent the harmful effects of iron salts, namely ferrous sulphate, on the gastrointestinal tract and on the natural microbiota of the digestive system. Figure 3 gives a diagrammatic representation of the difference between the oral intake of nano-encapsulated Fe drug and encapsulated Fe drug. It has the additional advantage of rendering enhanced bioavailability and absorption compared to conventional iron salts.
Nanotechnology for osteoporosis

Calcium deficiency is another common deficiency disorder prevalent mostly in developing countries due to low dietary intake of calcium [23]. Apart from maintaining bone strength and regeneration, calcium also plays a role in blood clotting, nervous system functioning, muscle contraction and cell signalling. Calcium deficiency is usually accompanied by vitamin D deficiency, since vitamin D is necessary for the absorption of calcium from the intestinal lumen into the blood [24]. Deficiency of calcium leads to decreased bone mineralization; when the calcium intake is low, calcium deposits present in the bones are utilized to replenish and maintain homeostasis in various body fluids and tissues. This leads to decreased bone density [25]. Osteoporosis is the thinning of bones caused by a variety of factors such as age, menopause, alcoholism, etc. Calcium and vitamin D deficient diet is also an important cause of osteoporosis. Especially in women, bone thinning starts at the onset of menopause and calcium deficiency can subsequently lead to osteoporosis. Ho et al. [26] conducted a 30-month study on 438 pre-menopausal Chinese women to identify the relation between menopause and bone changes. It was observed that an annual bone loss of 0.5% occurred in pre-menopausal women, 2%–2.5% in transitional women and 1.5% in post-menopausal women [26]. As shown in Figure 4 [27], the incidence of osteoporosis is generally higher in women than in men. Such bone density changes in menopausal women can be attributed to the decreased levels of oestrogen during menopause. Oestrogen, along with developing female secondary sexual characteristics, is also involved in maintaining the bone strength. Therefore, a fall in the levels of oestrogen during menopause increases the incidence of osteoporosis in women. Oestrogen treatment is recommended for post-menopausal osteoporosis management. However, oestrogen treatment has its own side effects and is not suitable for all women [28].

Conventional treatments for calcium deficiency include intake of calcium supplements containing calcium carbonate, calcium citrate or calcium phosphate. Calcium supplements have been found to slow down bone loss and also increase the axial bone density in menopausal women [29]. However, intake of calcium supplements, in turn, has some side effects like constipation and increased incidence of cardiac diseases and stroke. Pentti et al. [30] performed a study on 10,555 women in the age group of 52–62 years to analyse the effect of calcium and vitamin D supplementation on
coronary heart disease in women. It was observed that, among the 2723 subjects who regularly took calcium supplements, 513 women were diagnosed with coronary heart disease [30]. However, in another report, Shin and Kim [31] state that the association of cardiac diseases with calcium supplementation has still not been established due to absence of proper investigation and evidence. In either case, the side effects of artificial supplementation cannot be disregarded. The inevitable side effects of artificial supplements lead us to look into the field of nanotechnology to combat calcium deficiency. So far, only a few studies have been done regarding the application of nanotechnology in dealing with calcium deficiency.

Khajuria et al. [32] developed a nano-drug for osteoporosis that includes risedronate loaded onto zinc hydroxyapatite nanoparticles by an absorption method. Risedronate has a high affinity for bone and is used as the targeting moiety. Nano-sized hydroxyapatite has been found to increase the bioactivity of osteoblasts and also to increase bone regeneration [33–36]. The risedronate/zinc–hydroxyapatite particles were tested in ovariectomized rats/mice. Ovariectomy was performed to induce osteoporosis and prevent bone regeneration. In this study, the drug was injected intravenously to the rats. The drug was found to have anti-osteoporotic effect and to correct the bone loss in the ovariectomized rats. Thus, it was demonstrated that the drug not only prevented bone loss, but also stimulated bone growth. The combination of risedronate and hydroxyapatite proves to be a very effective way to deliver both calcium and phosphate, necessary for bone strength [33]. Further study can be done in this relation to find whether the oral route of delivery can be used for this formulation, as oral intake would be more favourable and easier for the patients. In addition, further clinical studies are strongly recommended to evaluate the potential side effects of such a formulation, as it has great potential to be commercially used for the treatment of osteoporosis.

Balasundaram and Webster [37] reported that bioactive compounds and drugs functionalized onto the surface of magnetic nanoparticles can be delivered directly to the osteoporotic bone (and not the healthy bone) and regenerate it. Magnetic nanoparticles can be transported to specific tissues or target site by an externally applied magnetic field and they lose their magnetism once the external magnetic field is removed. In this study, the authors functionalized biodegradable nanoparticles of hydroxyapatite with bioactive compounds that bind to bones of low biomass. These functionalized bioactive molecules were then placed on the outer surface of magnetic nanoparticles using various techniques, such as covalent chemical attachment. After binding to the osteoporotic bone, the nanoparticles deliver bioactive molecules to it to stimulate bone regeneration. What is more, the slow degradation of hydroxyapatite ensures...
that there is sustained long-term bone regeneration. One additional advantage provided by magnetic nanoparticles is that by using magnetic resonant imaging, the drug activity can be located and monitored. Further study is required to evaluate the attachment efficiency of magnetic nanoparticles to healthy and osteoporotic bones, determine release profiles and evaluate the toxic potential [37,38].

Critical Pharmaceuticals in collaboration with the University of Nottingham is developing a nano-enabled nasal formulation of teriparatide for the treatment of osteoporosis. Teriparatide is a parathyroid hormone and stimulates osteoblasts for bone growth [39]. Present treatment involves daily injection of teriparatide, which can be quite vexing. An absorption enhancer, Critical-Sorb, developed by the company itself using nanotechnology, is being used to formulate a nasal spray. The intranasal formulation will be non-invasive and more efficient than the conventional drug because of the enhanced absorption provided by nanotechnology (Information on the current status of the drug is unavailable and all the information discussed here is cited from news transcripts dated February 2012) [39]. The higher absorption and bioactivity can be attributed to the fact that a reduction in particle size greatly enhances the absorption and activity of the drug or molecule by increasing the overall surface-area-to-volume ratio. This is also confirmed by the findings of Erfanian et al. [3] in their study on the influence of nano-size reduction on calcium absorption and bioavailability in rats. Ovariectomized (OVX) and OVX-osteoporosis rats were fed with milk powder nano-fortified with calcium and the calcium absorption and bioavailability was investigated in both these groups. It was observed that the bone calcium and bone-breaking strength increased with the consumption of nano-fortified calcium. Calcium absorption in OVX and OVX-osteoporosis rats was 63.54% and 89.06%, respectively. The bioavailability of calcium from fortified and nano-fortified milk powders in OVX rats was 24.64% and 41.65%, respectively. The calcium bioavailability in the OVX-osteoporosis group from fortified and nano-fortified milk was reported to be 9.74% and 30.17%, respectively [3]. Another related study was conducted by Huang et al. [40] on the effect of nano calcium carbonate and nano calcium citrate on the bioavailability of calcium in OVX mice models and the toxic effects. It was observed that, at a nano calcium citrate and nano calcium carbonate concentration of 2.3 and 1.3 g/kg, there was no toxic effect. Instead, there was an increase in the serum calcium levels and the whole-body bone mineral density in the OVX mice. Thus, it appears that nano-size reduction dramatically enhances the absorption and bioavailability of calcium, as nanoparticles have greater ability to cross the wall of the intestine and also the way they are treated in the gastrointestinal tract gets altered [2]. However, with size reduction, the tendency of the particles to escape into unintended tissues or organs of the body increases and they can prove to be harmful to those organs or tissues. Hence, such side effects also need to be evaluated prior to the use of nano-sized calcium as a calcium supplement.

Tokudome et al. [41] conducted a study on the jaw bone mineral density and mechanical strength of osteoporosis rats administered with zinc-containing β-tricalcium phosphate nanoparticles (ZnTCP). OVX osteoporosis rats were injected with ZnSO₄ and ZnTCP powder dissolved in corn oil around the jaw bone. The bone mineral content was measured using an X-ray Computed Tomography scan and the bone mechanical strength was measured using the three-point bending technique. The bone mineral content was found to be 382.0 and 352.5 mg for ZnSO₄ and ZnTCP, respectively. The bone mechanical strength was found to be 98.9 and 157.0 N for ZnSO₄ and ZnTCP, respectively. ZnTCP induced sufficient bone formation because it is released slowly and in a controlled manner due to its low solubility in body fluids. ZnSO₄, on the other hand, is readily soluble due to which the injected ZnSO₄ particles immediately diffused into the tissues and were absorbed, dissolved or distributed throughout the body so that therapeutically significant levels on Zn could not be attained around the osteoporosis bone. Thus, it was found that ZnTCP nanoparticles could control the release of Zn and effectively induce osteogenesis in osteoporosis bones [41]. However, the harmful effects of ZnTCP nanoparticles need to be weighed against their therapeutic potential prior to their use as treatment for osteoporosis.

Kumar et al. [42] conducted an in vivo study on the efficacy of a prototype formulation based on layer-by-layer nano-matrix bearing kaempferol for the treatment of osteoporosis. Kaempferol is a flavanoid associated with good skeletal health [43,44]. It has been demonstrated to overcome the deleterious effect on bone density in oestrogen deficiency [43], to exhibit an anti-osteoclastogenic effect [45,46] and to promote osteoblast differentiation [42]. Layer-by-layer nano-matrix was prepared by the sequential absorption of two bilayers of sodium alginate and protamine sulphate over the CaCO₃ loaded kaempferol microparticles. OVX Sprague–Dawley rats were administered with kaempferol and formulated kaempferol for 12 weeks, at the end of which, the plasma and bone marrow kaempferol levels increased by 2.8-fold and 1.75-fold, respectively, in comparison to those in the rats given free kaempferol. The bone micro-architecture was well maintained up to 30 days after
withdrawal of formulated kaempferol. However, deteriora-

tion started within 15 days in the osteoporosis rats

administered with free kaempferol [47]. It is a novel for-
mulation to increase the bioavailability of flavonoids for

the stimulation of osteoblasts and maintenance of skele-
tal health. Kaempferol has the additional advantage that

it is completely non-toxic. Free kaempferol is poorly

bioavailable and, hence, is not used for the treatment of

osteoporosis. However, such a formulation can increase its bioavailability and might potentially treat

osteoporosis.

Various research works, thus, provide evidence for the

superior performance of nanomaterial-based drugs over

the conventional drugs for the treatment of osteopo-

rosis. Nanomaterial-based drugs even provide minimally

invasive therapies with remarkable results, not well-

accomplished by conventional therapies. This could also

replace the hormone therapy given to osteoporotic

women, which has serious side effects [28]. Similarly, in

the case of iron deficiency anaemia, nanotechnology-

based drugs could be used to achieve significant results

even in lower dosage and with minimum side effects.

The only hurdle faced is the evaluation of these pro-

posed concepts in human trials and subsequent com-

mercialization. Table 1 gives an overview of the recent

research work and patents that have proposed novel

nanotechnology-based treatments for iron deficiency

anaemia and osteoporosis.

**Nanotechnology in bone tissue engineering**

There has been an increase in the occurrence of bone

disorders and conditions and it is expected to double by

2020 [56]. These are mostly prevalent in communities

that lead a sedentary lifestyle in which aging increases

the incidence of obesity and hence, bone disorders.

Bone conditions are also prevalent in underprivileged

communities that have low dietary intake of calcium and

other vitamins and minerals. Autologous and allogenic

transplantation of bone tissue has traditionally been

used for bone repair and regeneration [56]. However,

due to the inherent limitations of both these methods,

efforts have been made to construct bone grafts using

the principles of tissue engineering, biomaterials, and

very recently, using nanotechnology [57]. Scaffolds

meant for bone tissue engineering should have the fol-

lowing properties: biocompatibility, remarkable porosity

and pore size of less than 100 μm, biodegradability, abil-

ity to hold growth factors, mechanical strength equiva-

lent to cortical bone and low cost [58]. The use of

nanomaterials to engineer scaffolds covers mostly all of

these requirements and, hence, is becoming widely pop-

ular for bone tissue engineering. Table 2 gives a list of

nanomaterials commonly used to develop scaffolds in

bone tissue engineering and also outlines the properties

that the nanomaterials impart to the scaffold. Figure 5

gives a schematic representation of various nanopar-

ticles used in bone tissue engineering and properties

impacted by them to the final scaffold. Figure 6 gives a

schematic representation of the steps involved in the

manufacturing of scaffolds for bone tissue engineering.

Deepthi et al. [59] and Mahoney et al. [60] review the

applications of chitin- and chitosan-based nanocompo-

sites for construction of scaffolds for bone tissue engi-

neering. Chitin and chitosan are ideal materials for bone

tissue engineering because of their biocompatibility, bio-

degradability, non-antigenicity, structural similarity to

glycosaminoglycans (major components of the extracel-

lular matrix of the bone), ability to form highly porous

scaffolds with interconnected pores, ability to enhance

bone formation and osteoconductivity. They can be

engineered into various forms like beads, microparticles,

gel, nanoparticles, scaffolds, nanofibres, etc. Different

biomaterials (summarized in Table 2) can be incorpo-

rated into the chitin and chitosan scaffolds to improve

their mechanical strength [61], biocompatibility [59,62]

and osteogenesis [61–66]. The following biomaterials are

mainly incorporated into the scaffolds: nano-

hydroxyapatite (nHAp), nano-bioactive glass ceramics, nano-silicon
dioxide (nSiO2), nano-titanium oxide (nTiO2), nano-zirconium
oxide (nZrO2) [67–72]. Hydroxyapatite is the most

ideal candidate for composite incorporation because it is

structurally similar to the natural bone components’

osteoconductivity and biocompatibility. Nano-sized

hydroxyapatite provides numerous advantages over

micro-sized hydroxyapatite because it easily gets dis-

dersed in the polymer matrix and forms regular pores

instead of irregular pores [73]. Uswatta et al. [54] de-

veloped injectable porous scaffolds from chitosan biopoly-

mer, nano-hydroxyapatite and sodium tripolyphosphate

to crosslink nano-hydroxyapatite and chitosan) by using

non-toxic coacervation and lyophilization techniques.

This was based on the hypothesis that incorporation of

nano-hydroxyapatite could improve the strength of the

scaffold and also stimulate osteoconduction, since it can

emulate the mineral structure of the bone. Murine osteo-

blast (OB-6) cell lines were used to evaluate the cell via-

bility, attachment and proliferation. The scaffolds

showed good results for cell proliferation for nHA in 2%

(w/v) chitosan. No toxic effects were found on the cells.

The authors also found that increasing nano-hydroxyap-

atite increased the cell adhesion and proliferation on the

scaffolds. The mechanical strength of the scaffolds can

also be increased by reducing the pore sizes [54]. The

findings of the above study are also substantiated by the

work of Sharma et al. [74], in which a bone regeneration
Table 1. Recent research work and patents that have proposed novel nanotechnology-based treatments for iron deficiency anaemia and osteoporosis.

| S. No | Area of research | Nanomaterial used | Details of research | References |
|-------|------------------|-------------------|---------------------|------------|
| 1.    | A ferritin core mimetic that corrects anaemia. | Iron nanoparticle | Tartarate modified, nano-disperse ferrihydrite was used to successfully deliver Fe(III) into the gastrointestinal system, without the risk of generation of free radicals, tested in murine models. This can be potentially used as a safe form of iron supplement. | [48] |
| 2.    | Nano-disperse iron for prevention and treatment of anaemia in weaning pigs. | Iron nano-suspension | A suspension of nano-disperse iron was obtained using the ultrasound method, during which the crystal lattice of iron breaks and increases the superfluous active surface atoms of iron. | [49] |
| 3.    | Nano mineral water | Iron nanoparticles | Nano-iron fortified mineral water increases the bioavailability and absorption of iron. | [50] |
| 4.    | Nano-sized ferric pyrophosphate as a food fortificant | Nano-sized particles of ferric pyrophosphate | Bioavailability of pyrophosphate is increased in the nano-form, since a reduction in size makes it readily soluble. | [13] |
| 5.    | Nano based iron composites | Nanocomposite | Iron oxides nanoparticles capped with a mixture of multivitamins such as folic acid, nicotinic acid and ascorbic acid developed for the treatment of anaemia in albino rats. | [22] |
| 6.    | Magnetite nanoparticles as a single-dose treatment for iron deficiency anaemia. | Magnetite nanoparticles | Magnetite nanoparticles coated with vitamin C can raise Hb levels from 7 to 14 g/dL within only 10 days of administration in albino rabbits. | [21] |
| 7.    | ColloidaLife™ Trace Minerals | Nanoparticles of boron, calcium, chromium, copper, iodine, iron, magnesium, manganese | Commercial supplement that is claimed to increase the bioavailability of trace elements in the body. | [51] |
| 8.    | Nanogel Trace Minerals | Calcium nanoparticles | An injectable product for minimally invasive surgery comprised of hydroxyapatite nanoparticles that fills bone defects with osteoconductive materials. | [52] |
| 9.    | Rejuvenate | Calcium and magnesium nanoparticles | Hydroxyapatite nanoparticles gel aimed to fill bone defects. | [53] |
| 10.   | Risedronate/zinc-hydroxyapatite based nano-medicine for osteoporosis. | Hydroxyapatite nanoparticles | Risedronate loaded onto zinc hydroxyapatite nanoparticles is shown to prevent bone loss and stimulate bone growth in ovariectomized rat models. | [32] |
| 11.   | Injectable porous nano-hydroxyapatite/chitosan/tripolyphosphate scaffolds with improved compressive strength for bone regeneration | Injectable porous scaffolds prepared from chitosan and nano-hydroxyapatite are demonstrated to have bone regeneration capacity owing to good osteoblast adhesion and proliferation. Incorporation of nano-hydroxyapatite is reported to increase the compressive strength of the scaffold. | [54] |
| 12.   | Nano enabled nasal spray for osteoporosis. | Nano-enabled intranasal formulation | Nano-enabled nasal formulation of teriparatide is reported to stimulate osteoblast cells. | [39] |
| 13.   | Influence of nano-size reduction on absorption and bioavailability of calcium from fortified milk powder in rats | Nano-sized calcium | Nano-size reduction of calcium is reported to have increased bioavailability than micro-sized calcium. | [3] |
| 14.   | Calcium bioavailability of nano-sized pearl powder | Nano-sized pearl powder | Nano-sized pearl powder is reported to have better calcium bioavailability, retention and absorption than micro-sized pearl powder. | [55] |
| 15.   | Effects of nano calcium carbonate and nano calcium citrate on toxicity in ICR mice and on bone mineral density in an ovariectomized mice model | Nano calcium carbonate and nano calcium citrate | Nano calcium carbonate and nano calcium citrate are reported to increase the serum calcium levels and whole body bone mineral density with no toxic effects in ovariectomized mice models. | [40] |
| 16.   | Absorption and bioavailability of nano-size reduced calcium citrate fortified milk powder in ovariectomized and ovariectomized-osteoporosis rats | Fortification of milk with nano-calcium citrate is shown to increase the bioavailability of calcium and bone stiffness and strength in ovariectomized and ovariectomized-osteoporosis rats. | [4] |

The scaffold was prepared using nano-hydroxyapatite, chitosan, gelatin and alginate by using a foaming method (without surfactant). Thus, nano-hydroxyapatite is an excellent means of bone regeneration, as it can easily be injected to the target site in a minimally invasive manner. However, in vivo studies are required to evaluate its side effects and to understand how well it can be applied for bone repair in humans. In another study, in vivo trials were performed in rabbits to test the bone formation ability of a nano-hydroxyapatite–fucoidan nanocomposite. The prepared nanocomposite was inserted in the tibia of osteometized rabbits. Slight bone formation was observed in the damaged bone [75]. However, the exact role of fucoidan in bone formation could not be established and needs further examination.

Nano-bioglass scaffold is another novel method of bone tissue engineering. Bioactive glass consists of SiO2, CaO, P2O5 and Na2O. A thin layer of hydroxyapatite or
hydroxycarbonate apatite forms on the glass surface, when it comes in contact with the biological fluids, which leads to the binding of soft tissues in the bone. Since the components of bioactive glass are those naturally found in the body, the glass is eventually absorbed and replaced by bone. The angiogenic potential of bioglass is enhanced by doping it with alum. It has been found that bioglass doped with alum exhibits higher cytocompatibility and biocompatibility than bioglass alone [93]. However, that study failed to bring out the advantages of bioglass over other biomaterials for use in bone tissue engineering. It also failed to evaluate the toxic effects of the components of the glass on surrounding cells and tissues and whether those are present in levels that are clinically recommended and safe for the human body or not.

Alginate is also used for construction of scaffolds owing to its biocompatibility, biodegradability, non-toxicity, non-immunogenecity, easy commercial availability and low cost. It can also be modified into desired forms like microcapsules, microspheres, hydrogels, sponges, fibres and foam. Alginate is incorporated with chitosan and/or various nano-sized biomaterials like hydroxyapatite, SiO₂, bioactive glass, calcium phosphate cement, etc. to improve its mechanical and osteoconductive properties [58].

### Challenges

The potential of nanotechnology to fight deficiency diseases like anaemia and osteoporosis is, no doubt, remarkable. However, it faces certain challenges that still
need to be overcome. One of the main challenges it faces, is that there is very little information available with respect to the fate of the nanomaterial once it enters the systemic circulation through any route. This makes it very challenging to develop nano-based drugs or targeting moieties as the information pertaining to it is limited. Usually, drugs are tested on animal models like rats, mice or rabbits, but the same physiology and drug interaction is hardly applicable to the human body [94–99]. This increases the chances of failure of the drug or might result in the drug reaching untargeted sites. Also, this limitation makes it difficult to quantify the correct dose of the drug that is required to bring out the desired effect that is safe and tolerable for the body. For example, in the case of nano-Fe drugs for treatment of anaemia, the correct quantity of drug needs to be administered as a high dose, resulting in harmful side effects and potentially causing accumulation of iron in the body which is, again, detrimental. Another challenge is the transport of the nano-drugs across the compartmental boundaries like endothelial and epithelial barriers of vessels, placental and intestinal walls, etc. Another type of barriers faced by the drug are in the form of degrading enzymes, scavenging phagocyes, abnormal flow of blood and hydrostatic pressure at target sites and molecular and ionic efflux pumps that can expel the drug out of the cell [100]. Thus, detailed knowledge of the interaction of nano-drugs with these barriers is important to design a successful, barrier-resistant drug.

Another challenge related to the manufacturing of nanomaterials for drug delivery, is the large-scale production. There is a need to scale up the laboratory or pilot technologies for consistent and reproducible production and commercialization. It is difficult to modify or control the desired properties of these nanomaterials at large scale. The main challenges to scale up the process include agglomeration, low concentration of nanomaterials and chemical processes. For instance, maintaining the size and composition of nanomaterials at laboratory scale is possible, whereas it is a challenge at large-scale production of these nanomaterials. Although a number of drug delivery technologies have been patented, the commercialization of these technologies is still at its early stage. The main reason of the more limited commercialization is the gap between academic research and pharmaceutical companies because this type of research has been mostly carried out by researchers in universities. Therefore, there should be partnership between universities and pharmaceutical companies so that these technologies can reach into the market [101,102].

Along with the augmented research and development work on drug delivery, safety concerns about the uses of these technologies are also emerging. Nanoparticles have distinct properties from corresponding bulk particles, such as surface characteristics, physicochemical properties and crystal structure. Hence the distinct toxicity of nanoparticles [103,104]. There are various ways of entry of nanoparticles into the human body, such as

Figure 5. Schematic representation of the various biomaterials used in conjugation with chitosan- and chitin-based scaffolds and the respective properties they impart to the scaffold.
dermal, gastrointestinal and respiratory routes. The smaller size and larger surface area of nanoparticles than their corresponding bulk materials are the main factors that contribute to the toxicity of nanomaterials. Due to the small size of nanoparticles, they can easily pass through the biological barriers and membranes and can cause toxicity at various sites in the body, i.e. genotoxicity, carcinogenic potential and oxidative stress in organelles, mitochondrial stress and cell-wall disruption \cite{105–107}. Therefore, the safety and the possible impact of these nanomaterials should not only be considered for the affected population, but should also cover the entire manufacturing and disposal processes \cite{102}. Proper regulatory guidelines must be established for the assessment and evaluation of nanotechnology-based drugs and products.

**Conclusions**

Technologies to encapsulate micronutrients to increase their bioavailability, safety and stability are either already under development or active research is underway pertaining to such technologies. Such technology can not only be utilized to overcome the limitations of the conventional drugs and nutrient supplements, but they can also provide better results than the conventional drugs. Currently, it is necessary to develop low cost and safe nano-based drugs or formulations to overcome deficiency diseases, highly prevalent in developing and underdeveloped countries. It is imperative to bring nanotechnology based drugs from the laboratory into large scale manufacturing and conduct clinical trials for the same so that they can be put to use as quickly as possible.
Nanotechnology provides abundant advantages and benefits which must be exploited for the betterment of mankind.

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