



# Bone regeneration in osteoporosis: opportunities and challenges

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## Abstract

Osteoporosis is a bone disorder characterised by low bone mineral density, reduced bone strength, increased bone fragility, and impaired mineralisation of bones causing an increased risk of bone fracture. Several therapies are available for treating osteoporosis which include bisphosphonates, anti-resorptive agents, oestrogen modulators, etc. These therapies primarily focus on decreasing bone resorption and do not assist in bone regeneration or offering permanent curative solutions. Additionally, these therapies are associated with severe adverse events like thromboembolism, increased risk of stroke, and hypocalcaemia. To overcome these limitations, bone regenerative pathways and approaches are now considered to manage osteoporosis. The bone regenerative pathways involved in bone regeneration include wntless-related integration site/ $\beta$ -catenin signalling pathway, notch signalling pathway, calcium signalling, etc. The various regenerative approaches which possess potential to heal and replace the bone defect site include scaffolds, cements, cell therapy, and other alternative medicines. The review focuses on describing the challenges and opportunities in bone regeneration for osteoporosis.

**Keywords** Osteoporosis · Bone regeneration · Scaffolds · Cements · Cell therapy

## Introduction

Osteoporosis is a disorder of bones characterised by low bone mineral density (BMD), reduced bone strength, and increased bone fragility of the bones causing higher risk of bone fracture [1]. This bone disorder frequently remains undiagnosed until a low-trauma fracture of spine, hip, pelvis, wrist, or proximal humerus is identified, which may often lead to hospitalisation. Osteoporosis is a silent disease until the incidences of fractures appear frequently and cause secondary health issues [2]. The annual osteoporotic fractures are expected to rise by 50% in the year 2025 [3]. A greater than 87% rise is anticipated in individuals of age group 65 to 74 years [4]. The risk of osteoporotic fracture is around 40 to 50% in women and 13 to 22% in men [5]. During twenties, the bone mass of an adult human reaches its peak level. Thereafter, it starts to decline as the speed of bone resorption crosses the speed of bone formation. Although the bone mass decreases by 1% in ageing humans, it decreases by 3%

in postmenopausal women [6]. As the bone structure gets damaged due to uncontrolled formation of regulators, such as hormones and local factors, the individual becomes more susceptible to osteoporosis [7].

The primary cause of osteoporosis is ageing, whereas there are several secondary causes which include genetic diseases like cystic fibrosis and glycogen storage diseases, endocrine diseases namely central obesity and diabetes, gastrointestinal disorders namely gastric bypass and malabsorption, haematological disorders, i.e. thalassemia and haemophilia, neurological disorders which include epilepsy and multiple sclerosis, and rheumatological and autoimmune disorders like rheumatoid arthritis and systemic lupus [8]. Life style modifications, namely vitamin D and calcium deficiency, high salt intake, alcohol, and smoking, significantly increase the risk of osteoporosis [9].

Osteoporosis is classified into two types that are primary osteoporosis and secondary osteoporosis. Primary osteoporosis is further classified as type 1 and type 2. Type 1 primary osteoporosis is also called as postmenopausal osteoporosis which results from oestrogen deficiency [10]. Type 2 primary osteoporosis is termed as senile osteoporosis which is due to ageing and affects both men and women [11]. Secondary osteoporosis is associated with secondary causes like drugs or diseases which cause bone mass reduction, an

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etioloical factor that can be clearly identified and distinguished [12].

There are multiple pharmacological therapies available for treatment of osteoporosis like anti-resorptive agents, vitamins, and calcium, but these conventional therapies come with proven undesirable side effects (Table 1). For example, bisphosphonates may lead to odd fractures of the bones due to excessive rigidity caused by the treatment, and hormonal therapies may lead to severe adverse events like thromboembolism. The current therapies are more inclined towards decreasing bone resorption and do not focus on offering a permanent solution for osteoporosis.

Although autogenous bone grafts are the gold standard for reconstruction of large bone defects, it has demerits such as lack of sufficient transplantable materials, donor site morbidity, resorption of implanted bone, and inflammation [13]. Synthetic grafting materials and allografts are useful to overcome these demerits of autogenous bone grafts. However, they are limited due to lack of osteoconductivity and immunogenesis [14].

Regenerative therapies have the potential ability to heal and replace damaged organs and tissues due to factors like age, diseases, trauma, as well as congenital defects. The current drawbacks of bone grafting, such as lack of availability of the donor and severe immune-related complications, can be bypassed by means of regenerative medicines [23]. Thus, novel therapeutic strategies based on the advances in molecular and cellular biology aiming to regenerate damaged bones and tissues have been developed recently and are under further research for assessment of fate of bone regenerative approaches. Therefore, this article primarily focuses on the bone remodeling, pathways of bone regeneration, and various bone regenerative approaches that can be incorporated for the management of osteoporosis.

## Process of bone regeneration in osteoporosis

### Bone remodeling cycle

Bone is an extremely dynamic tissue that constantly undergoes modelling and remodeling through the activation of cells including osteoblasts, osteoclasts, and osteocytes [24]. The osteoclasts originate from haematopoietic precursor cells and are primarily responsible for bone resorption, whereas the osteoblasts originate from mesenchymal stem cells (MSCs) and assist in bone formation [25]. Osteocytes, terminally differentiated osteoblasts, are located in mineralised bone and participate in controlling time and site of bone remodeling [26]. The bone remodeling is a five-phase process involving activation, resorption, reversal, formation, and termination.

The activation phase mainly involves stimulating bone remodeling signal due to the expression of receptor activator of nuclear factor kappa-B ligand (RANKL) in osteocytes at damaged bone sites, which enhances recruitment, differentiation, and fusion of osteoclast precursors to form osteoclasts. Osteocytes convert the mechanical strain signals into biological signals to initiate bone remodeling. Parathyroid hormone (PTH) assists in maintaining calcium homeostasis and can trigger bone remodeling by binding to receptors on osteoblasts [27]. In resorption phase, osteoclast enrolment takes place at remodeling site. The osteoblast releases cytokines and matrix metalloproteases for stimulation of osteoclastogenesis. This leads to unmineralised osteoid degradation and formation of attachment sites for osteoclasts [28]. During reversal phase, the osteoclast facilitated bone resorption is completed, and the bone prepares itself for osteoblast-mediated bone formation via removal of collagen debris by mononuclear cells [29].

The differentiation of osteoblast progenitor cells and secretion of bone growth facilitating molecules like type I collagen, proteoglycans, lipids, alkaline phosphatases, and integrin-binding proteins forms the key highlight of formation phase [30]. In later stage, remodeling is terminated when resorbed bone is completely substituted by newly formed bone in quantitatively equal portion. The mineralisation of osteoid is the final step in bone formation phase, in which hydroxyapatite crystals grown in matrix vesicles deposit on type I collagen. Osteoclasts undergo apoptosis in few weeks, while osteoblast apoptosis takes few months. The increasing rate of bone remodeling will lead to loss bone density [31]. There is an imbalance between bone resorption and bone genesis in case of osteoporosis which can be resolved by targeting pathways which promote bone regeneration.

### Pathways of bone regeneration in osteoporosis

There are multiple pathways which assist in bone regeneration via restoring the balance between osteoblasts and osteoclast functions, thereby stimulating new bone formation at the defect site.

Wingless-related integration site (Wnt)/ $\beta$ -catenin signalling pathway participates throughout the bone healing process as it is known to regulate wide range of cell-fate decisions. Wnt signalling through  $\beta$ -catenin participates in healing of bone fractures by promotion of osteoblastogenesis and osteoblast functions [32].

Notch signalling pathway is a ligand receptor signalling pathway which promotes cell proliferation, differentiation, survival, and cell-fate decision [33]. This pathway gets activated upon interaction of notch ligands with its receptors. It is a potential pathway with direct osteoinductive effects on bone cells called osteoblasts [34].

Table 1 Insights to present therapies used for osteoporosis

Drugs	Formulation	Mechanism	Side effects	Reference
<b>Anti-resorptive therapy</b>				
<b>Bisphosphonates:</b>	Oral	Inhibits bone resorption by binding to surfaces where bone resorption occurs	Osteonecrosis of the jaw, renal complications, atypical femoral fractures, and gastrointestinal upsets	[15]
<b>Nitrogen-containing (e.g. ibandronate, alendronate, zoledronate, risedronate, and pamidronate)</b>	Parenteral			
<b>Non-nitrogen-containing (e.g. etidronate)</b>	Oral	Increases osteoblast activity and decreases osteoclast activity	Thromboembolism and increased risk of myocardial infarction	[16]
<b>Strontium ranelate</b>	Oral	Mimics effects of oestrogen on bone	Causes thromboembolic events and strokes in postmenopausal women with cardiovascular disease	[17]
<b>Selective oestrogen receptor modulators (SERMs):</b>	Oral			
<b>Raloxifene and arzoxifene</b>	Parenteral intranasal oral	Inhibits bone resorption	Long term use increases risk of prostate cancer, less effective in postmenopausal women	[18]
<b>Calcitonin</b>	Oral	Promotes osteoclast apoptosis	Increased risks of breast cancer, stroke, and thromboembolism	[19]
<b>Oestrogen replacement therapy: 17<math>\beta</math>-estradiol, esterified and conjugated oestrogens</b>	Transdermal			
<b>Monoclonal antibodies: Denosumab</b>	Parenteral	Binds to receptor activator of nuclear factor-kappa B ligand (RANKL) and inhibits osteoclasts	Hypocalcemia, osteonecrosis, and cystitis	[20]
<b>Parathyroid hormone (PTH) treatment</b>	Parenteral	Promotes osteoblastogenesis and survival of osteoblasts	Cannot be administered beyond 2 years	[21]
<b>Teriparatide</b>	Oral	Stimulates osteoblast activity, decreases reactive oxygen species	Needs to be combined with other management therapies	[22]
<b>Vitamins and natural products</b>				
<b>Vitamin D, calcium, magnesium, green tea, citrus fruits</b>	Oral			

Bone morphogenetic protein (BMP)/transforming growth factors- $\beta$  (TGF- $\beta$ ) pathway is another key pathway participating in bone regeneration. BMPs belong to superfamily of TGF- $\beta$  which is essential in osteogenesis [35]. BMPs can activate endochondral formation of bone in mice; however, it is difficult to evaluate the activity of BMPs in foetus and adults [36]. Among BMPs, BMP2 is of interest in the field of clinical research as it has shown to be effective in bone regeneration [37]. BMP2 facilitates bone regeneration by stimulating MSCs and osteoblast progenitor cells which leads to callus formation [38, 39].

Phosphatidylinositol 3-kinase/threonine protein kinase/mammalian target of rapamycin pathway is an important mitogenic signalling pathway for cellular processes such as cell growth, motility, and proliferation. It has a critical regulatory function and participates in bone formation and remodeling [40].

Mitogen-associated protein kinase pathway is a vital link between the surface and nucleus of the cell and regulates proliferation, differentiation, migration, and cell death which is critical in bone formation [41]. However, its impact on osteoblasts is controversial; some suggest that it is stimulatory, while others suggest that it is inhibitory [42].

Platelet-derived growth factor (PDGF) signalling is an extracellular factor which controls many cell functions in the skeleton through its mitogenic, proliferative, and

angiogenic properties leading to rise in numbers of MSCs [43]. Recent studies indicate that pharmacological inhibition of PDGF receptors reduces the proliferation of MSCs without affecting osteoblastic differentiation [44].

Insulin-like growth factor (IGF) signalling is an important signalling pathway involved in proliferation and differentiation of osteoblasts. IGF1 and IGF2 are the only members of IGF family with similar properties and biological characteristics, and both are expressed in osteoblasts [45]. IGF2 is abundantly found in the bones. Both the IGFs differentiate osteoblasts, express collagens and non-collagenous proteins, and stimulate bone matrix deposition [46].

Fibroblast growth factor (FGF) pathway maintains proliferation and differentiation of fibroblast osteoblasts during the formation of bone cells and regulates endochondral and intramembranous signalling in osteoprogenitor cells [47].

Calcium has a structural role in bone formation since calcium signalling pathway enhances osteoblast differentiation [48]. During the process of bone remodeling, calcium is constantly released into the extracellular environment in the form of free ions which are available for various biological activities of osteoprogenitors and osteoblasts [49]. Figure 1 describes the bone regeneration pathways.

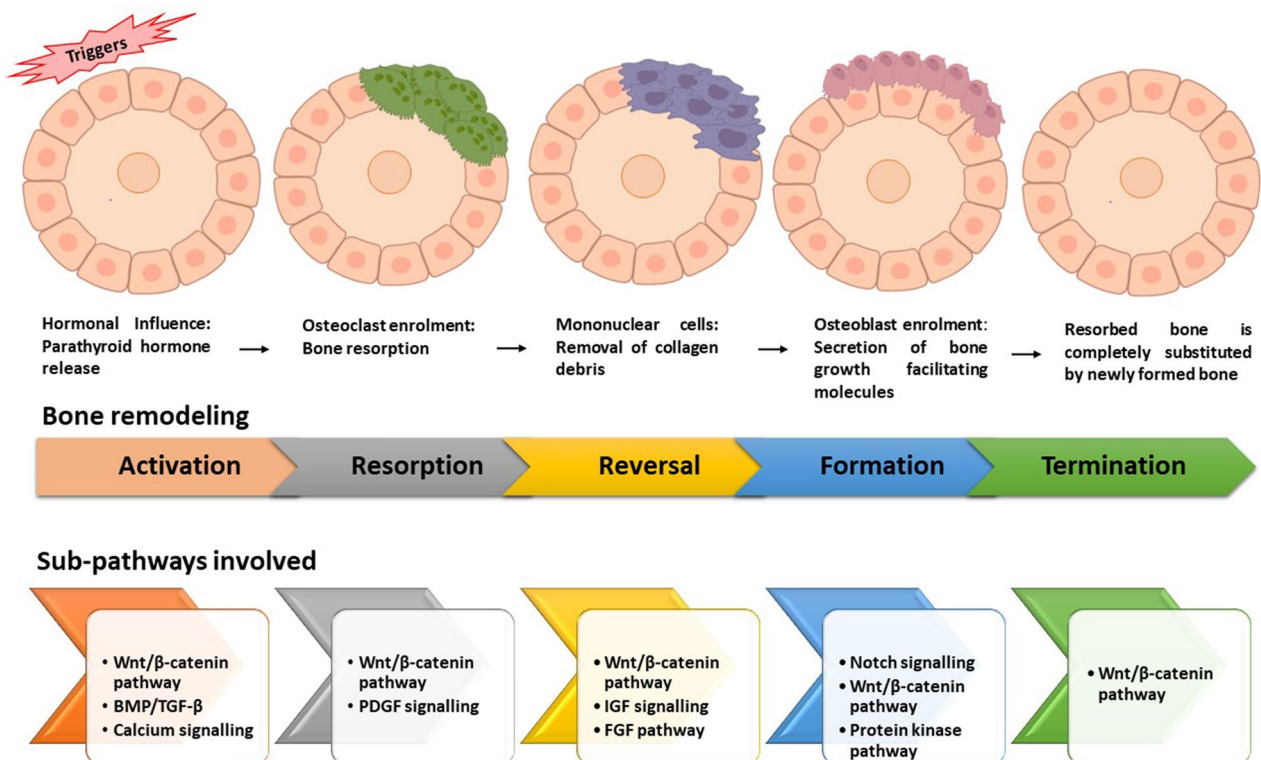


Fig. 1 Bone remodeling and bone regeneration pathways

## Approaches for bone regeneration

The reconstruction of the severely defected and injured bones is a highly technical challenge that needs synthetic substitutes with osteoinductive and osteoconductive properties along with sufficient mechanical properties [50]. Several approaches for the management of osteoporosis are being investigated which includes scaffolds, cements, cell therapy, and other alternative medicines.

### Scaffolds with potential of bone regeneration

An ideal scaffold for bone regeneration should be biocompatible, form chemical bond with the host bone, have interconnected pore structure, a surface appropriate for the attachment of osteogenic cell, exhibit properties similar to the host bone, and possess potential to be commercially producible and sterilisable [51]. Figure 2 describes the advantages of scaffolds in bone regeneration.

#### Bioactive glass scaffolds

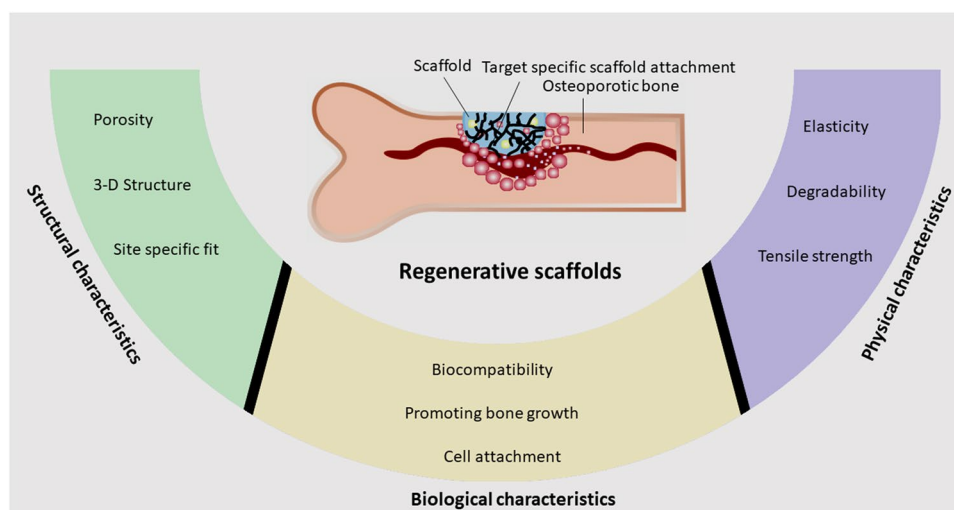
Bioactive glass is an ideal material for scaffolds due to rapid binding with the bone and having biodegradable nature. Bioactive glasses are attractive in bone tissue engineering due to osteoinductive and osteoconductive properties and promote bone growth via proliferation of osteoprogenitor cells [52]. Bioactive glass can be made of natural polymers, such as collagen, chitosan, silk, or alginate. It can also be prepared from synthetic polymers, such as polyesters and copolymers [53]. The *in vivo* and *in vitro* effectiveness of bioactive glass mainly depends on the composition and the pore structure of the glass scaffolds. Although direct bonding occurs between the scaffold and the new bone, bioactive glass scaffolds have limitations. Toughness and mechanical reliability of these

scaffolds limit their use in loaded bone repair. However, a composite with biocompatible coating polymer may aid in overcoming this limitation [54]. Chlanda et al. coated bioactive glass with polylactide and polycaprolactone polymers for improving mechanical properties, porosity, and adhesiveness [55].

Alendronate is orally administered drug used in osteoporosis due to its capabilities of inhibiting bone resorption. However, systemic administration of alendronate leads to poor bioavailability and increased toxicity. Alendronate-loaded amino-modified bioactive glass scaffolds were prepared using powder processing technique as a novel delivery approach. The scaffolds could successfully promote bone growth by upregulation of alkaline phosphatase and down-regulation of RANKL. The histomorphometric assay indicated twofold increase in area of newly formed bones which confirms early bone regenerative abilities of amino-modified bioactive glass scaffolds in ovariectomized rats [56]. On a similar line, zoledronic acid-loaded mesoporous bioactive glass fabricated with polycaprolactone were prepared by robocasting. The scaffolds showed good *in vitro* and *in vivo* biocompatibility with osteoblast and osteoclast cells. *In vivo* studies performed in osteoporotic sheep model suggested promotion of new bone formation via inhibition of osteoclast activity [57].

Combination of calcium and silica glass scaffolds fabricated with strontium were suggested to enhance expression of various osteogenic and angiogenic markers in osteoporosis. The polymerase chain reaction assay showed Runt-related transcription factor-2 (RUNX-2), and vascular endothelial growth factor (VEGF) were upregulated, thereby indicative of osteogenic effects. The increased ratios of bone volume to total volume and trabecular thickness compare to conventional calcium silica scaffold confirmed *in vivo* bone regenerative abilities of the strontium calcium silica scaffold [58]. Similar observation was reported in a study

**Fig. 2** Advantages of bone regenerative scaffolds



where mesoporous strontium bioactive glass scaffolds were prepared. The results suggested that new bone formation was promoted via upregulation of RUNX-2, alkaline phosphatase, and bone gamma-carboxyglutamate protein [59]. These strategies are thus well-suited alternatives for delivery strontium ranelate.

### Hydrogel scaffolds

Hydrogels are promising candidates in tissue engineering due to their abilities of promoting biomineralisation and osteointegration. Additionally, hydrogels have improved mechanical strength and aid in providing suitable environment for bone regeneration. Hydrogels assist in tailoring scaffold geometry, controlling drug release and enhancement of porosity [60].

Alginate hydrogel scaffold loaded with  $\beta$ -estradiol and BMP2 for sustained release and bone regenerative abilities in osteoporosis was prepared by electrospinning. Macroscopic analysis of calvaria indicated that the defect was filled, and the new bone formation was confirmed by 22% higher amount of adipose and connective tissue in the defect area [61]. In another study, thermoresponsive alginate hydrogel loaded with microsphere of  $\beta$ -estradiol, plasma-rich growth factors, and BMP2 was administered locally for regeneration of calvaria bone defects in osteoporotic rats. The histological and histomorphometric analysis showed that all components exhibited a synergistic effect which lead to improvement in bone regenerative abilities possibly due to upregulated osteocalcin and collagen type I [62].

The composite structure of biomineral combines the advantages of both organic and inorganic materials. Mineralised hydrogels easily mimic mineral phase of native bones. Mineralised hydrogel was prepared using nano-hydroxyapatite, sodium carbonate, and polyacrylic acid [63]. The live dead assay showed that bone marrow MSCs improved cell viability when they were cultured on mineral hydrogel matrix. The F-actin intensity was measured after 3-day incubation with bone marrow MSCs. The mineral hydrogels showed 1.46 folds greater F-actin intensity in bone marrow MSCs than control suggesting early maturation of bone marrow MSCs and enhancement of bone cell functions. The over expression of multiple osteogenic markers precisely alkaline phosphatase (1.79 folds), RUNX-2 (1.65 folds), osteopontin (1.39 folds), and osteocalcin (1.42 folds) is indicative of bone regenerative abilities of mineral hydrogel scaffold. The increased oestrogen levels and bone volume fraction confirm the bone regeneration. The hydrogel showed excellent stability, biocompatibility, and osteoconductive abilities [63].

Tissue scaffolds with three-dimensional nanofibrous scaffolds were prepared using gelatin, nano-hydroxyapatite, and polylactic acid by electrospinning technique. BMP2 was later immobilised on the scaffolds and cultured with bone

MSCs for 14 days to assess change in levels of osteogenic markers. The upregulation of alkaline phosphatase, RUNX-2, and osteocalcin confirmed bone regenerative abilities of the scaffold. The micro-computed tomography suggested that new bone formation completely filled defects in rat cranial bone defect model of osteoporosis [64].

### Bone cements

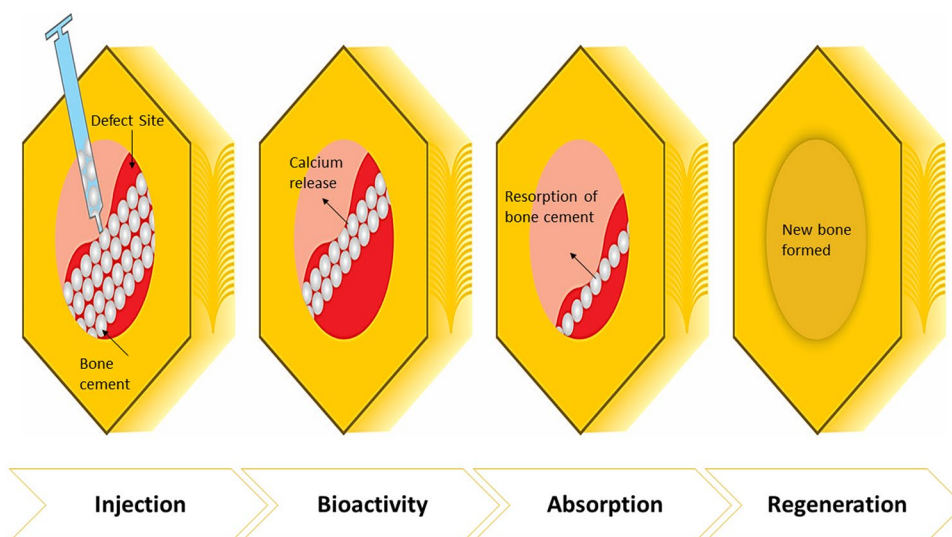
Bone cements are biomaterials consisting of powder phase and liquid phase, which are implanted as a paste at defect site and easily set at body temperature. An ideal bone cement should have mechanical properties resembling the bone of the individual permitting cellular ingrowth and supporting new bone formation; it should degrade with time as the new bone regenerates and replaces this substitute. Additionally it should have ease of handling, injectable nature, and appropriate in vivo setting times and should not disintegrate upon contact with body fluids [65]. Bone cements are advantageous for development of regenerative alternatives since they offer curative solution, perfectly fit implant site, and provide assured mechanical support. Figure 3 describes the mechanism of action of bone regenerative cements.

### Calcium phosphate bone cements

Calcium phosphate cements are extensively used for the treatment of bone defects due to biological performance, including biodegradability, biocompatibility, osteoinductivity, osteoconductivity, acceptable in vivo setting time, and interactions with cells [66]. Calcium phosphate degrades in the body causing release of calcium and phosphate ions leading to alteration of the bioactivity, including proliferation, adhesion, and formation of new bone via the osteoblasts. The release of calcium ions increases the local concentration of the ions promoting bone mineral formation on the surface of calcium phosphate cement. It is crucial in adhesion of cells and formation of tissue as it adsorbs the proteins from the extracellular matrix onto its surface [67]. Calcium phosphate cements are superior in performance as it can be used as an injectable in surgical procedures, which makes the surgeries minimally invasive [68]. Hydroxyapatite, a natural form of calcium phosphate, is extensively used in bone regeneration and comprises the largest part of the inorganic component of the bones. Tricalcium phosphate is a widely studied calcium phosphate along with hydroxyapatite; it has a high bone resorption rate and is useful in increasing biocompatibility. Whitlockite is a ceramic made of calcium phosphate and also contains magnesium ions; it is one of the most abundant minerals found in human bone [69].

The study reported development of composite bone cement by adding calcium silicate to calcium phosphate cement that did not affect chemical structure of later and

**Fig. 3** Mechanism of action of bone cements



had very little effect on the compressive strength and setting time. It was found to improve cell proliferation and enhance *in vitro* and *in vivo* bioactivity of the cement. Although this composite cements can aid in the degradability and bioactivity, the study did not confirm regenerative abilities of the cements [70].

Trace elements or trace metals are minerals present in biological tissues in relatively minor quantity [71]. A variety of trace elements like lithium, strontium, zinc, fluorine, iron, boron, copper, manganese, magnesium, and selenium are osteoprotective which promote activity of osteoblasts and suppress action of osteoclasts [72]. Copper acts as a co-factor for antioxidant enzymes, whereas magnesium is an essential co-factor for regulating calcium metabolism [73]. Research investigated fracture healing potential of lithium-doped calcium phosphate bone cements. Lithium activated Wnt/ $\beta$ -catenin signalling pathway which enhances bone formation and leads to increase in bone mass, thereby effectively healing the fracture. The presence of lithium in calcium phosphate cements also lowered tumour burden and suppressed *in vivo* myeloma development in osteoporotic rats. It showed excellent bioactivity, biocompatibility, osteoconductivity, and osteointegration abilities [74]. Strontium-doped calcium phosphate cements also indicated similar results where upregulation of alkaline phosphatase was identified as key factor for promoting bone growth [75].

Drug-loaded calcium phosphate cements for localised treatment of osteoporosis are thought of as key candidates for targeting specific skeletal areas prone to osteoporotic fractures. Alendronate-loaded calcium phosphate cement was reported for localised osteoporosis treatment. Alendronate was released gradually in a sustained manner over a period of 21 days. A significant increase in BMD and bone volume was reported in ovariectomised rats owing to inhibition of bone resorption by alendronate [76].

### Acrylic bone cements

Acrylic bone cements are another type of cements which have been used in the field of orthopaedics since decades. The marketed products of acrylic bone cements consist of two phases, i.e. polymethylmethacrylate (82–89% w/w), an inorganic radiopacifying agent such as zirconium dioxide or barium sulphate (10–15% w/w), and benzoyl peroxide (0.5–2.6% w/w) [77].

Poly methyl methacrylate bone cement is used for management of osteoporosis-induced fractures. The clinical applications are restricted due to poor performance and weak binding to the bone. To overcome these limitations, mineralised collagen-loaded poly methyl methacrylate bone cement was prepared. Improvement in alkaline phosphatase and bone marrow MSCs activity was noted which indicated bone formation. *In vivo* studies performed in rabbit model indicated significantly increased osteoblast activity and new bone area which can be correlated with bone regeneration potential of developed cement [78].

With use of poly methyl methacrylate bone cement, there is a risk of bone stiffening. Hence, poly methyl methacrylate was combined with freshly harvested bone marrow cells from sheep. The modified cement showed reduced Young's modulus indicating high porous nature of cement which would overcome bone stiffening. There was an improvement in mechanical properties like viscosity, setting time, and hardening time noted for modified cement. The use of bone marrow cells can also assist in bone regeneration; however, there was no experimental evidence drawn from the study pertaining to regeneration potential of developed cement [79].

### Cell therapy

An inflammatory stimulus causes a cascade of inflammatory and regenerative events for repair and healing of

injured bone. It includes the release of cytokines, allocation of the immune cells to the wounded site, oedema and inflammation of the soft tissue, osteogenic progenitor cell differentiation, BMP release, callus formation, and bone remodeling [80].

Mesenchymal stem cells (MSCs) are common precursors for adipocytes and osteoblasts. The properties on MSCs isolated from bone marrow of healthy individual compared with postmenopausal women with osteoporosis suggest that the intrinsic properties of MSCs are disturbed in those with osteoporosis [81]. The age-related reduction in proliferation of MSCs also suggest that MSC therapy may be useful in osteoporosis [82].

Cell therapies utilising MSCs, which possess self-differentiating and self-renewal properties, are the solution to many problems associated with bone fracture or bone diseases. The migration of endogenous and exogenous MSCs to the injured site of the bone is necessary for bone healing. Inflammatory mediators secreted by immune cells chemokines and TGF- $\beta$ 1 regulate the allotment of endogenous MSCs. Immediate use of MSCs post the injury cause reduction in local and systemic inflammatory response, whereas MSCs administered at intermediate periods after the injury participate in healing and repair of the bone by differentiation into osteoblasts and chondrocytes. This causes stimulation of local endogenous allotment of osteoprogenitor cells [83]. MSCs can be delivered to the wounded area through local or systemic injections. Systemic MSC injections are preferable when the injuries are at multiple sites with complex nature, and local injections are preferred for single injury.

MSCs were incorporated in a calcium alginate gel matrix and injected into femur of ovariectomized rabbits. Post 8 weeks, 50% increase in trabecular thickness and upregulation of alkaline phosphatase activity was noted in treated

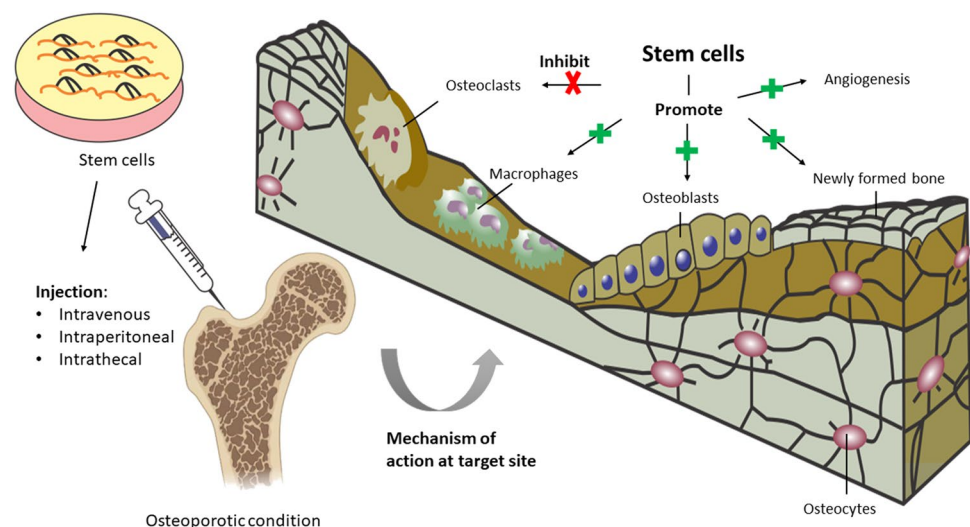
rabbits which indicates new bone formation [84]. Ocarino et al. injected MSCs isolated from healthy rat bone marrow into femur of ovariectomized rats where significant enhancement in trabecular thickness was noted. The green fluorescence protein labelling technique successfully confirmed MSCs lining surface of newly formed bone at defect site [85].

The main limitations of MSC-based therapy is the complexity of cell types to differentiate into osteogenic lineage upon treatment, and these therapies are also considered to be immune evasive. Thus, cell therapy has promising possibility in the treatment of osteoporosis, but it comes with many intrinsic obstacles, such as lack of bone homing ability in MSCs. Additionally, there is always an uncertainty regarding the fate of cell post-implantation [86]. Advance research needs to be conducted to evaluate and confirm the safety and effectiveness of cell therapy in osteoporosis. Figure 4 describes overview of cell therapy as bone regenerative approach.

### Alternative medicine

The elements present in deep sea water (DSW) and the phytoconstituents, such as phytoestrogens, form an integral part of alternative approaches for managing osteoporosis [87]. Sea water obtained from a depth of more than 200 m is referred to as DSW and is rich in various minerals, such as calcium, potassium, chromium, selenium, vanadium, zinc, and magnesium [88]. It is also known to stimulate production of osteoblasts and osteoclasts. Sodium chloride present in DSW improves the alkaline phosphatase activity to stimulate bone growth. In vivo studies indicate that combining DSW with soluble silicon promotes proliferation of osteoblasts and enhances osteogenesis-related expression of RUNX2 genes and alkaline phosphatase [89]. DSW has a

**Fig. 4** Stem cell therapies for bone regeneration in osteoporosis





potential in treatment of osteoporosis by causing significant rise in osteoblastic proliferation rate which was observed in cytotoxicity assay and increase in osteogenic differentiation markers like BMP2, RUNX-2, osteocalcin, and osteopontin. Microcomputed tomography images also indicated formation of new bone in ovariectomized SAMP8 mice after use of DSW [90]. Therefore, DSW can be a potential agent for the overall improvement of bone health in osteoporosis.

Oestrogen is involved in the life cycle of the bone cells, and it also regulates the activity and expression of several inflammatory cytokines in bone remodeling. Many cases of osteoporosis occur due to lack of oestrogen in postmenopausal women and individuals with hypogonadism. Hence, plant-derived oestrogen, also referred to as phytoestrogens, can be useful in the treatment of osteoporosis [91]. Phytoestrogens are similar to mammalian oestrogens but have a milder effect on sensitive tissues, such as uterus and breast. Some common phytoestrogens include lignans found in flax-seeds/linseed, isoflavones found in soy, and flavonoids [92]. *Epimedium brevicornum maxim*, plant rich in phytoestrogen flavonoids, was studied for its bone preventive abilities in postmenopausal women. Eighty-five participants successfully completed the 2-year-long double-blind clinical trial. The osteocalcin level was enhanced by 10.7% upon treatment with *Epimedium brevicornum maxim* (four capsules daily). Osteocalcin is a protein synthesised by osteoblasts which is indicative of osteogenic maturation and new bone formation [93].

Many herbs, known as kidney tonics and used in traditional medicines, were tested and proven to enhance bone metabolism. In a randomised clinical trial, *Herba epimedii*, *Fructus ligustri lucidi*, and *Fructus psoraleae* (10:8:2) were boiled and extracted. The *in vitro* results indicated osteoblastic and anti-osteoclastic properties of the extract. This extract also showed promotion of BMD in ovariectomized rats [94]. In another study, alcoholic

extract of *Antrodia camphorata* were proven to promote formation of bone cells and prevent bone loss, both *in vitro* with proteoblasts and *in vivo* in ovariectomized mice [95]. Granules prepared from *Spinacia oleracea* were administered orally in drill hole fracture model of rats that exhibited bone regeneration potential. One hundred twenty-two percent enhancement in bone volume/tissue was observed in treated group. Twofold increase in RUNX-2, BMP2, osteocalcin, and collagen 1 were reported which indicates bone regenerative potential of *Spinacia oleracea* granules [96]. Table 2 summarises various herbs known to promote bone regeneration in osteoporosis. The data suggest the effectiveness of herbs; however, further research needs to be conducted to confirm the safety and efficacy of herbs in osteoporosis.

## Challenges and opportunities

The multiple mechanisms involved in bone regeneration process make development of bone regenerative therapies challenging. To establish a curative treatment for osteoporosis, it is essential to understand the mechanisms involved and identify a suitable signalling pathway capable of promoting bone regeneration process. Hence to bypass this challenge, development of molecular targets promoting bone regeneration is being looked upon as an upcoming area in the current research scenario. Additionally, multi-modal targeting strategies having scientific rationale can also be thought of as promising alternatives for offering bone regenerative potential. However, to witness clinical translation of such multi-modal targeting or combination therapies, it is crucial to illustrate an improvement in efficacy and safety of proposed treatments. Theoretically, the principles and benefits of regeneration are encouraging but only few products have reached clinical trials (Table 3).

**Table 2** Commonly used herbs for promoting bone regeneration in osteoporosis

Herb	Phyto active	Mechanism	Reference
<i>Withania somnifera</i> (root)	Withaferin A, syringic acid, gallic acid, naringenin	Increase in RUNX-2 expression, stimulation of osteoblast production	[97]
<i>Cissus quadrangularis</i> (aerial plant)	Catalpol, eugenol, resveratrol, rutin	Stimulate oestrogen signalling and Wnt/ $\beta$ -catenin signalling pathway	[98]
<i>Tinospora cordifolia</i> (stem)	Beta-ecdysone, syringin, carvacrol, berberine, magnoflorine	Increase in RUNX-2 expression and enhancement of osteoblast precursors	[99]
<i>Moringa oleifera</i> (leaf, seed)	Myricetin, quercetin, and kaempferol	Decreases PTH	[100]
<i>Nigella sativa</i> (seed)	Beta-amyryn, campesterol, beta-sitosterol	Increases alkaline phosphatases and prevents calcium loss	[101]
<i>Butea monosperma</i> (bark)	Cajanin, isoformononetin, cladrin, medicarpin	Manages osteocalcin level	[102]
<i>Ginkgo biloba</i> (leaf)	Kaempferol, quercetin, isorhamnetin, ginkgolic acid	Increased the expression of genes promoting osteoblastogenesis	[103]

**Table 3** Clinical trials for osteoporosis focusing on bone regeneration

NCT number	Study title	Conditions	Interventions	Phase
NCT01074723	B-cryptoxanthin and phytosterols on bone remodeling and cardiovascular risk factors	Postmenopausal osteoporosis	b-cryptoxanthin and phytosterols	1
NCT00100607	Safety and efficacy of AAE581 in postmenopausal women with osteoporosis	Osteoporosis	AAE581	2
NCT00715676	Phase 2 safety and efficacy study of a vitamin D compound (DP001) in postmenopausal women with low bone mineral density (2MD-3H-2B)	Osteoporosis	Placebo, DP001	2
NCT00693667	Study of PH3 for the prevention of osteoporosis in postmenopausal women (PH3)	Postmenopausal osteoporosis	PH3	2
NCT00018447	Combination osteogenic therapy in established osteoporosis	Postmenopausal osteoporosis	PTH 1–34, MFPSR-fluoride	2
NCT04501354	Evaluation of clinical and bone density improvement after implantation of allogenic mesenchymal stem cell from umbilical cord on osteoporosis patients	Osteoporosis	MSCs	2
NCT02061995	Phase 2a study on intravenous infusion of autologous osteoblastic cells in severe osteoporosis	Severe osteoporosis	PREOB® Intravenous Infusion	2
NCT00471237	A phase II study evaluating SB-751689 in postmenopausal women with osteoporosis	Osteoporosis	Ronacaleret, Teriparatide, Alendronate	2
NCT00452439	A study of Actonel for the prevention of bone loss	Osteoporosis	Actonel, calcium, Vitamin D	3
NCT00500409	Safety and efficacy of OSTEOFORM (rhPTH [1-34]) in increasing bone mineral density in osteoporosis	Osteoporosis	Osteoform, Shelcal	3

There are various agents, such as chemokines, cytokines, growth factors, and other signalling molecules, which can be vital in bone regeneration due to their abilities in regulating bone formation. However, the use of these agents for bone regeneration is hindered due to difficulty in achieving site-specific targeting and maintaining effective therapeutic concentrations at target site [104]. Gene therapy is an alternative which can overcome these obstacles in delivery. Although gene therapy is not tested in humans, animal models prove the efficacy of this novel drug delivery [105]. Exogenous genetic material has also been introduced for modification and correction of cell function and differentiation. Transcription of genes related to critical regulators, such as BMP, osteoprotegerin, PTH, and targeted drug delivery in bone remodeling has proven to be beneficial in osteoporosis. However, further studies need to be conducted to identify the safety and efficacy of gene therapy as a novel approach in bone regeneration in osteoporosis. As microRNA (miRNA) plays a role in epigenetic regulation of the disease and bone metabolism, the targeted activation or inactivation of the miRNA of specific bones can be another molecular therapy to improve the osteoanabolic responses [106].

Functionalization of biomaterials with peptides or natural biomolecules which supports bone regeneration and would assist in enhancement of targeting efficiency can be thought

of as another opportunity in developing bone regenerative solutions for osteoporosis [107]. Designing of rational combination therapies capable of promoting bone growth could help in better management of osteoporosis [108]. The development of simpler and industrially scalable manufacturing techniques for regenerative approaches would help witness early bench to bed translation [109].

## Conclusion

The adverse events and complications associated with use of present treatments are avoided, and the effectiveness of therapies in osteoporosis is significantly improved by means of regenerative medicines. The regenerative therapies for osteoporosis include use of scaffold, cement, cell therapy, and alternative medicines. The regenerative therapies are promising for offering curative solutions which would improve the quality of long-term treatment for osteoporosis. The personalised medicine aspect to osteoporosis has opened new avenues which requires identification of key bone regenerative signalling pathways and genes specific to each individual. There is a need for further research to be conducted to evaluate the safety and efficacy of regenerative therapies for osteoporosis to witness clinical translation.

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## Declarations

**Ethics approval and consent to participate** Not applicable.

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