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Strontium-doped hydroxyapatite and its role in osteogenesis and angiogenesis

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ABSTRACT For the past 50 years, hydroxyapatite (HA) has been widely used in bone defect repair because it is the main inorganic component of the mineral phase of a human bone. Extensive preclinical and clinical studies have shown that strontium (Sr) can safely and effectively help prevent and treat bone diseases, including osteoporosis. These findings have resulted in the concept of integrating Sr and HA for bone disease management. The doped Sr can improve the physicochemical properties of HA and enhance its angiogenic and bone regeneration ability. Nevertheless, no study has reviewed the design strategy of Sr-doped HA (Sr-HA) to understand its biological roles. Therefore, in this article, we review recent developments in Sr-HA preparation and its effect on osteogenesis and angiogenesis *in vitro* and *in vivo* along with key suggestions for future research and development.

KEYWORDS: hydroxyapatite, strontium, biological mechanism, osteogenesis, angiogenesis

Introduction

The clinical treatment of serious segmental bone defects caused by trauma or bone tumors is challenging. Moreover, seeking an alternative to autologous bone grafting is one of the major goals of bone tissue engineering. Bone engineering has been extensively studied by researchers over the past few decades. Consequently, many artificial bone materials have been developed. HA has garnered extensive attention from researchers because this main inorganic component of bone tissues (Jarcho *et al*., 1977; Zhan *et al*., 2005; Kutikov *et al*., 2015; Deville *et al*., 2006; Li *et al*., 2013; Poddar *et al*., 2023) is the most important bone repair/regeneration material owing to its excellent biocompatibility and osteoinductive properties. However, its poor mechanical properties and slow degradation limit its application to clinical practice (Rezwan *et al*., 2006). Researchers think that modifying HA may solve the mentioned problems.

Currently, the biological properties of HA-based materials can be improved by several methods, including doping other elements, heat treatment, and material coating (Shavandi *et al*., 2015; Shi *et al*., 2020; He *et al*., 2021; Boanini *et al*., 2010). Adding certain metal ions can improve the physicochemical properties of HA and enhance its antibacterial, angiogenic and bone regeneration capacity (Turhan *et al*., 2023). For example, strontium (Sr), magnesium (Mg), and zinc (Zn) ions can promote bone regeneration by regulating osteoblast and osteoclast activity.

Mg is the fourth most abundant element in the human body, with 60% of magnesium deposited in the bones (Laskus and Kolmas, 2017). Mg alloys began to be used in orthopedics and blood vessels in the mid-nineteenth century (Walker *et al*., 2014). Mg forms bone by promoting osteoblast differentiation. He *et al.,* demonstrated that Mg metal enhanced the viability and osteogenic differentiation of human bone marrow-derived stromal cells (hBMSCs) (He *et al*., 2016), as did Yang *et al.,* (Yang *et al*., 2010). It is reported that Mg2+ replacement of Ca2+ in HA occurs only to a limited extent, up to 10 at.% (Bigi *et al*., 1996; Yasukawa *et al*., 1996). Despite the limited substitution, the doping of Mg leads to an increase in the solubility of HA (Landi *et al*., 2008), which may be related to

Abbreviations used in this paper: BMSC, bone marrow-derived stromal cell; Sr-HA, strontium-doped hydroxyapatite.

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TABLE 1

ADVANTAGES AND DISADVANTAGES OF THE PREPARATION METHODS OF STRONTIUM-DOPED HYDROXYAPATITE (SR-HA)

Method	Advantage	Disadvantage
Chemical precipitation method	1. Certain interactions exist among the groups 2. Good permeability and adsorption 3. Certain inducement	1. Unknown results 2. Low product purity
Wet process preparation	1. High crystallinity and stability 2. Resistant to corrosion 3. Biocompatible	1. The product is not stable 2. Slow reaction rate and large product particle size
Hydrothermal method	1. No cytotoxicity 2. Good biocompatibility	1. Thermal stability performance becomes poor 2. Easy to decompose
Wet microwave synthesis	1. Able to fuse with natural bone 2. Increased cell growth rate 3. Strontium content is controllable 4. Shorten reaction time	Currently not widely used

the decrease in crystallinity. Meanwhile, the morphology of HA is changed to a spherical shape (Berg *et al*., 2020).

Zn is an essential trace element in the human body, involved in DNA and RNA replication, protein synthesis, and bone metabolism (Prasad, 1995). Zn promotes bone metabolism by increasing osteoblast activity and collagen synthesis, as well as inhibiting osteoclast formation (Yamaguchi and Gao, 1998; Kishi and Yamaguchi, 1994). Zn2+ probably replace up to 20% of the Ca2+ in the HA lattice (Boanini *et al*., 2010). Since it causes defects in the structure, it makes the lattice more susceptible to disruption (Gutsalova *et al*., 2021), which makes Zn-doped HA less soluble than conventional HA (Osorio *et al*., 2014; Hu *et al*., 2012). Zn-doped HA crystals are irregular and form agglomerates, usually in the form of rods (Hu *et al*., 2012).

The trace element Sr is present in the human body at 0.008% - 0.01%, which is much lower than that of Ca. However, Sr has a strong affinity for bone, especially in metabolically active tissues (Dahl *et al*., 2001). Sr has a dual effect on bone: stimulation of bone formation and inhibition of bone resorption (Saidak and Marie, 2012; Hassan *et al*., 2023). On the one hand, Sr activates signaling pathways such as OPG/RANKL/RANK, NFκB to inhibit osteoclast activity; on the other hand, Sr enhances alkaline phosphatase (ALP) activity, collagen synthesis, and the formation of osteogenic markers to promote bone production (Huang *et al*., 2020; Zarins *et al*., 2019; Zarins *et al*., 2018; Rybchyn *et al*., 2011). Because Sr2+ (0.12 nm) has an ionic radius similar to Ca2+(0.099 nm), it can replace Ca in the HA structure throughout the compositional range (Boanini *et al*., 2010). A study showed that Sr2+ enters the HA lattice and enhances the mechanical strength (Geng *et al*., 2016). Landi *et al.,* confirmed the increase in the solubility of Sr-HA (Landi *et al*., 2007). Dai *et al.,* analyzed HX-BGC, a bioactive glass with 1.6 % Sr, was in the form of bands and plates (Dai *et al*., 2021b). Although Sr can replace up to 100% of Ca (Frangopol *et al*., 2016), in one study it was found that the HA structure was maintained at Sr molar ratios of 2% and 4% in the compound, while it disappeared at other different Sr molar ratios (Nagyné-Kovács *et al*., 2018). In addition, the Sr content has an effect on osteogenesis. When the Sr concentration exceeds a certain threshold, there will be toxic inhibition (Liu *et al*., 2016). Almeida *et al.,* demonstrated that the optimal concentration range of Sr is 1-10 mM, and within this range, Sr can effectively enhance the proliferation and activity of preosteoblasts, and promote the maturation of osteoblasts into osteocytes (Almeida *et al*., 2016).

Therefore, in this review, we systematically introduce the preparation method of Sr-HA and provide insights into the effects of implanted Sr-HA on osteogenesis and angiogenesis. Furthermore, the role of Sr in osteogenesis and vascularization in bone repair has also been discussed in detail.

Preparation of Sr-Doped hydroxyapatite

HA is an important component of the human skeleton. Because of its low solubility in physiological environments, as it is a bioactive material with a high osseointegration capacity that does not induce inflammatory reactions on direct contact with hard tissues. However, the properties of HA can be easily improved. The Ca2+ in the structure can be replaced by Sr2+, Zn2+, Cu2+, Fe2+, Ag+, Mg2+ and other ions (Dapporto *et al*., 2022; Ungureanu *et al*., 2023), so Sr can change the HA lattice to modify its biological properties in order to overcome any potential disadvantages; therefore, researchers have integrated Sr and HA. Sr-HA can be prepared by several methods, and four methods, namely chemical precipitation, wet processing, hydrothermal preparation, and wet microwave synthesis, have been discussed below. Furthermore, their advantages and disadvantages are presented in Table 1.

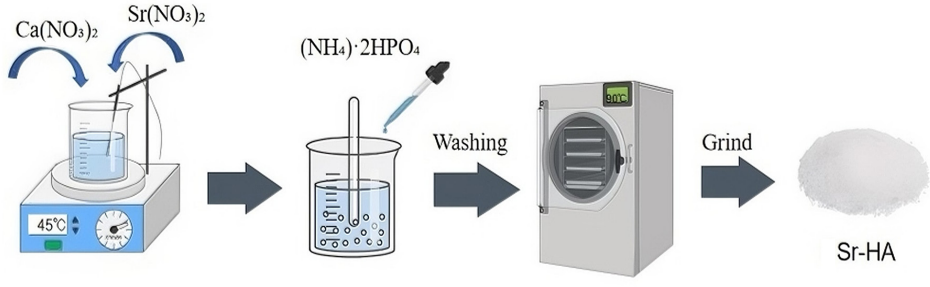

Fig. 1. Preparation of strontium-doped hydroxyapatite (Sr-HA) by the chemical precipitation method. (Created with BioRender.com).

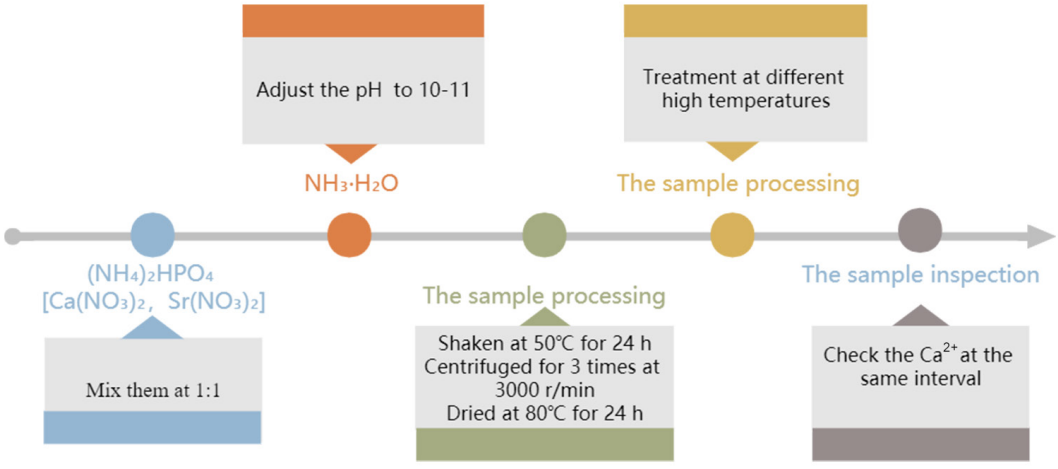

Fig. 2. Preparation of strontium-doped hydroxyapatite (Sr-HA) by the wet process method. (Created with BioRender.com).

Chemical precipitation method

Chemical precipitation method is to use the reaction between the reactant solution, by controlling the pH value of the solution, the solution precipitates. The crystallization process of precipitation is carried out by controlling the reaction rate, temperature, and aging time. It is the most widely used and simplest method to prepare Sr-HA. Scaffolds prepared by this method exhibit good dispersion and ably meet the requirements of tissue engineering.

In this method, $(CaNO_3)_2 \cdot 4H_2O$ and $(NH_4)2HPO_4$ are used as the main reactants. First, 0.5 mol/L $Ca(NO_3)_2$ and $Sr(NO_3)_2$ solutions are mixed at 45°C together. After the reaction, the pH of the solution is adjusted to 10-11 with $(NH_4)2HPO_4$. After, precipitates are filtered, washed with anhydrous ethanol to remove impurities, vacuum-filtered and -dried, and ground to obtain Sr-HA powder (Fig. 1). The use of Sr/[Ca+Sr] and [Ca+Sr]/P at atomic ratios of 0.5 and 1.67, respectively, can yield 0.5-nm Sr-HA (Zhu *et al*., 2022; Catros *et al*., 2010). Ehret and Maqbool *et al.,* (Ehret *et al*., 2017; Maqbool *et al*., 2021) prepared Sr-HA with good biocompatibility by this method without changing the phase composition and crystallinity of HA. The reaction mechanism of Sr-HA preparation by chemical precipitation is as follows:

(NH4) 2HPO4+NH4OH+ (10-α) Ca (NO3) 2+αSr (NO3) 2→H2O+SrαCa10-α (PO4) 6 (OH) 2+NH4NO3, α≤10

Wet process method

In this method, $(NH_4)_2HPO_4$, $[Ca(NO_3)_2$, and $Sr(NO_3)_2]$ solutions are mixed at a molar ratio of 1:1, and the molar ratio of Sr/(Ca+Sr) is set to 1:100.The solution is then kept in a shaker incubator at 50°C for 24 h, centrifuged thrice at 3000 r/min, and dried at 80°C for 24 h. The obtained blocks are then ground and sieved. Part of the powdered samples are cold-pressed into blocks at 200 kg pressure and heat treatment at 300°C, 600°C, and 900°C for 1 h (Fig. 2) (Pan *et al*., 2009). Li *et al.,* (Li *et al*., 2019) used this method to prepare Sr-HA, which exhibited good biocompatibility and stability, but its preparation time is long and the reaction speed is slow. It is not widely used in industrial production.

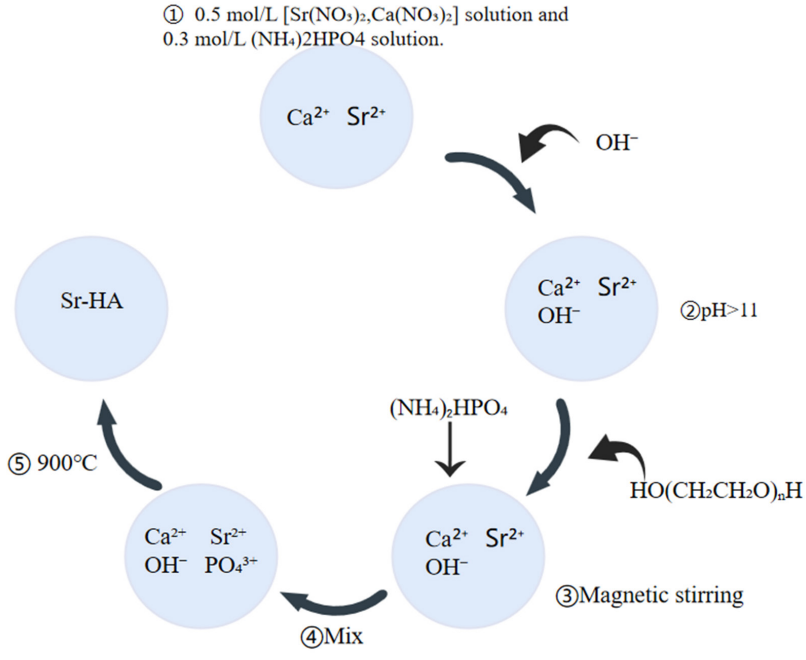

Fig. 3. Preparation of strontium-doped hydroxyapatite (Sr-HA) by the hydrothermal method. (Created with BioRender.com).

Hydrothermal method

In this method, a 0.5 mol/L $[Sr(NO_3)_2, Ca(NO_3)_2]$ solution and a 0.3 mol/L $(NH_4)2HPO4$ solution are prepared. The pH values of these Ca and Sr solutions are adjusted to >11 using concentrated ammonia and to >10 using a phosphorus solution. Next, a certain amount from both solutions is mixed thoroughly, followed by the addition of 0.4 g of polyethylene glycol and magnetic stirring to dissolve polyethylene glycol. Under the stirring condition, the $(NH_4)2HPO_4$ solution is added slowly in a dropwise manner to maintain the n (Ca+Sr)/n (P) atom ratio at about 1.67, and again the mixture is stirred well. Then, it is transferred to a teflon-lined stainless steel hydrothermal kettle and heat-treated at 900°C. The product is then washed with water, followed by an ethanol wash, and filtered. Then the powdered product is dried at 80°C overnight (Fig. 3) (Li *et al*., 2022; Donazzon *et al*., 1998). The reaction mechanism of Sr-HA

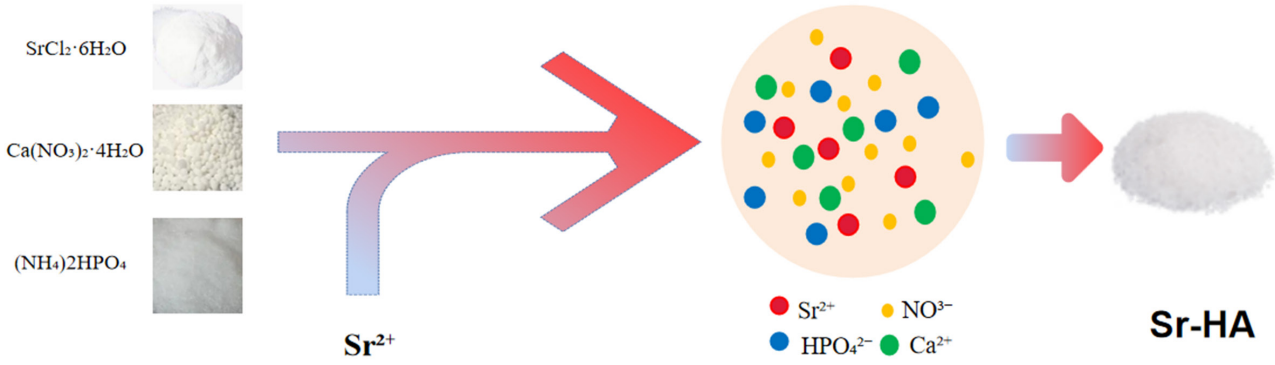

Fig. 4. Film formation of strontium-doped hydroxyapatite (Sr-HA) coating deposited on the surface by microwave. (Created with BioRender.com).

preparation by hydrothermal method is as follows:

(10-x)Ca2++xSr2++6PO43++2OH-→Ca10-xSrx(PO4)6(OH)2

Wet microwave synthesis method

This method is characterized by the simultaneous generation of heat at different depths of the material being heated. Compare the other three methods, this method allows for faster and more uniform heating (Fig. 4).

The crystals of SrCl2, $Ca(NO_3)_2{\cdot}4H_2O$, $(NH_4)_2HPO_4$ are used in this method. First, $[Ca(NO_3)_2{\cdot}SrCl_2]$ and $(NH4)_2HPO_4$ solutions of the 0.5 mol/L are prepared and pH is adjusted to 11 using concentrated ammonia. Next, the $(NH_4)_2HPO_4$ solution is rapidly poured into the $[Ca(NO_3)_2{\cdot}SrCl_2]$ solution and kept in a heating water bath at 80°C. The reaction is performed in a microwave oven with the power set to 800 W. The slurry is allowed to stand for a while, followed by filtration. The sediment is washed with distilled water and then with anhydrous ethanol, dried at 800oC, and finally ground (Yu *et al*., 2017; Agrawal *et al*., 2018). However, the resulting product is unstable; hence, this method is not used widely.

Osteogenic effects of Sr and Sr-HA

Owing to the similarity between Sr and Ca, Sr exhibits osteogenic properties similar to that exhibited by Ca, which can increase the levels of runt-related transcriptional factor 2 (RUNX2) and further stimulate Ca sensing receptor (CaSR), thereby inducing mitogen-activated protein kinase phosphorylation and activating cell signaling pathways, and ultimately increasing primary osteoblast formation (Gu *et al*., 2013). Sr promotes collagen secretion in osteoblasts via CaSR, thus increasing the levels of osteopontin (OPN), ALP, bone sialoprotein, salivary protein, and osteocalcin during osteoblast differentiation (Li *et al*., 2015). Thus, when Sr-HA is implanted, HA forms a strong bond with bone tissues. After coming in contact with body fluids, HA is degraded over time, thereby increasing the local Sr2+ concentration at the action site. The released Sr2+ exerts its osteogenic properties, inducing osteoblast differentiation (Fig. 5) (Wan *et al*., 2020). Brennan observed a dose-dependent increase in RUNX2/CBFA1 levels in human primary osteoblasts after Sr-Ran administration for 10 days. Moreover, OPN mRNA levels were increased by 50% after Sr-Ran (1 mM) administration (Brennan *et al*., 2009). Additionally, Sr promotes bone matrix synthesis (Barbara *et al*., 2004). Frasnelli observed that the cultures of rat skull osteoblasts exposed to Sr-Ran showed an increased synthesis of collagen I, a marker of osteoblast differentiation (Frasnelli *et al*., 2017).

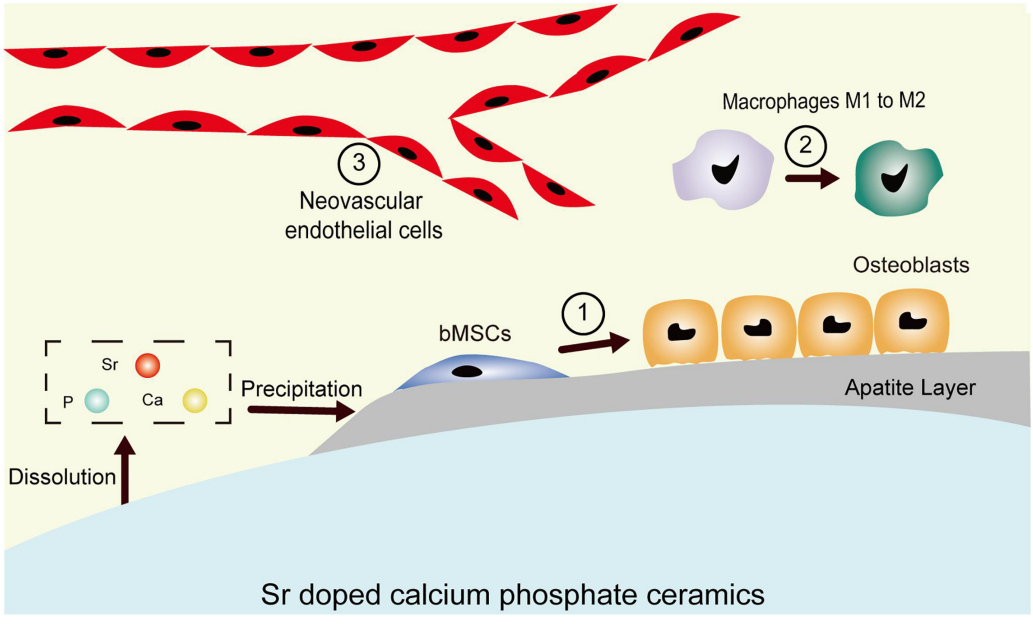

Fig. 5. The degradation and precipitation reaction of bioactive strontium-doped hydroxyapatite (Sr-HA) regulates local ion concentrations and influences surrounding physiological processes, including osteoblast differentiation. Abbreviations: bMSC, bone marrow-derived stromal cell ; Sr, strontium. Source: Wan *et al*., 2020. Licensed under CC BY.

Effects of Sr on osteoclasts

Osteoclasts are specialized cells within the bone tissue responsible for bone resorption or the breakdown of bone matrix. They play a crucial role in the dynamic process of bone remodeling, which involves the continuous removal of old bone tissue and the formation of new bone. Osteoclasts achieve bone resorption by secreting enzymes and acids that dissolve the mineralized matrix, making room for the subsequent action of bone-forming cells like osteoblasts.

Studies have shown that Sr promotes osteoclast apoptosis, inhibiting their proliferation and differentiation, thereby reducing bone resorption (Bonnelye *et al*., 2008). Owing to their similar atomic and ionic properties, both Ca and Sr are agonists of CaSR (Luo *et al*., 2018). Sr probably acts on CaSR to affect the apoptosis of mature osteoclasts (Fig. 6) (Neves *et al*., 2017; Borciani *et al*., 2022). Furthermore, Sr regulates inflammatory states to promote the osteogenesis of BMSCs and accelerate the inhibition of macrophage RAW264.7 differentiation (Li *et al*., 2016). In another study, consistent with *in vitro* results, Capuccini proved that the activity and differentiation of MG-63 were increased and the differentiation of osteoclasts was inhibited by Sr (Capuccini *et al*., 2008), confirming that Sr incorporation reduced the immune response to the material, thereby promoting bone regeneration *in vitro* (Lee *et al*., 2021). Additionally, Sr reduces the levels of carbonic anhydrase II and glass agglutinin receptors in osteoclasts, thereby inhibiting cell differentiation and reducing osteoclast resorption by up to 66% (Baron and Tsouderos, 2002). The above studies suggest that Sr promotes osteogenesis.

Effects of Sr-HA on osteogenesis

The chemical and physical properties of Sr and Ca are similar; however, Sr has a larger ionic radius than Ca (112 vs. 99 pm). The partial substitution of Ca by Sr results in higher solubility compared with solubility in the presence of Sr-free HA owning to unit cell enlargement (Wan *et al*., 2020). Thus, Sr binds to hyaluronic acid by adsorption on mineral surfaces. Moreover, the surface of Sr-HA is active and biodegradable, which forms bone-like apatite on its surface because of continuous dissolution and precipitation (Kołodziejska *et al*., 2021). Osteogenesis, the highly regulated process of bone formation, is a complex and crucial aspect of the skeletal system's development and maintenance. It involves the intricate interplay of various cellular components, including osteoblasts and osteoclasts, as well as the deposition and resorption of bone matrix. Many studies have shown that, compared with HA, Sr-HA promotes the osteogenesis of BMSCs, reduces the bone-healing period, and enhances implant bone fusion (Table 2). Furthermore, Sr concentration in the range of 3%–7% stimulates osteoblast activity and differentiation, and the 1% Sr concentration affects osteoblast proliferation (Boanini *et al*., 2011). These results imply that the doping of the trace element Sr promotes osteoblast activity and differentiation, whereas it inhibits osteoclast differentiation, exerting a potential positive effect *in vivo* (Gu *et al*., 2013).

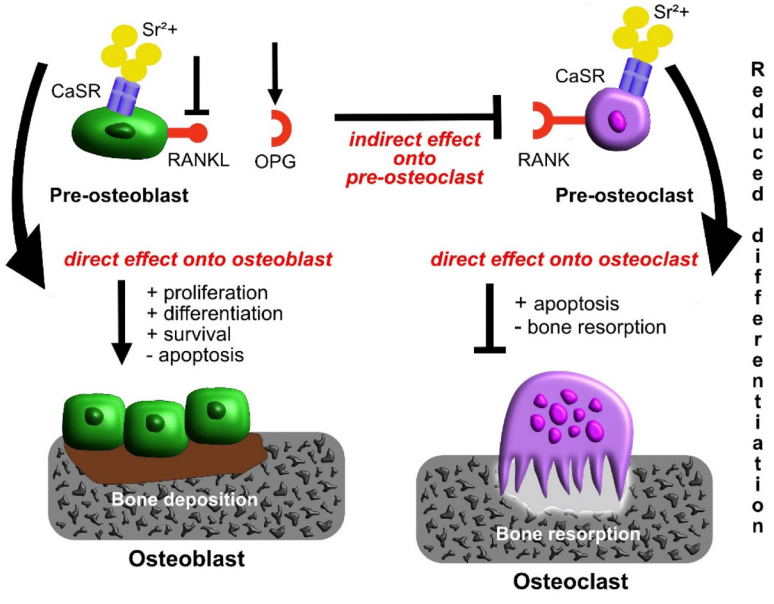

Fig. 6. Schematic diagram of influence of strontium (Sr) administration on osteoblasts and osteoclasts and their crosstalk. Source: Borciani *et al*., 2022. Licensed under CC BY 4.0.

Effects of Sr and Sr-HA on blood vessels

Pro-vascularization function of bone repairing biomaterials

Fracture healing is a gradual process of restoring normal bone form and function. It is important to realize that in addition to the development of new bones, the regeneration of bones involves blood vessel remodeling, implying that cells in new bone tissues require nutrients and excrete waste materials via blood vessels; in other words, the ideal bone tissue material should exhibit the function of pro-vascularization (Mao *et al*., 2009). The endothelial cells are pivotal in angiogenesis; therefore, the prerequisite for biomaterial graft vascularization is good affinity and compatibility with endothelial cells (Chen *et al*., 2006). Angiogenesis is mainly affected by vascularizing growth factors, and among all known members of vascular endothelial and angiogenic growth factors, vascular endothelial growth factor (VEGF) is the most specific pro-angiogenic factor, which plays a central regulatory role in physiological and pathological angiogenesis (Hirschi and D'Amore, 1997).

Notably, biomaterials are widely used in the treatment of bone defects and bone regeneration, where immune modulation, especially the abatement of inflammation, and the pro-vascularizing effect of biomaterials are crucial, where macrophages and neutrophils have a key role in the process of tissue-engineered angiogenesis. It is widely accepted that M2 macrophages are associated with promoting angiogenesis, while M1 macrophages have a comparatively weaker effect on angiogenesis (Wang *et al*., 2017). Li *et al.,* (Li *et al*., 2021) prepared Sr-HA nanofibrous gelatin scaffolds to study Sr-mediated regulation of neutrophil polarization and subsequent effects on angiogenesis and macrophage polarization. Sr-HA-doped gelatin scaffolds released by Sr were found to polarize neutrophils to an N2 phenotype and act on subsequent macrophages and effector cells, thereby promoting angiogenesis and tissue regeneration. Liu *et al.,* (Liu *et al*., 2023) summarized the mechanisms associated with Sr in bone regeneration (Fig. 7): Sr induces macrophage differentiation and promotes vascular regeneration.

Mature osteoblasts and endothelial cells can express VEGF and basic fibroblast growth factor (bFGF). Sr-HA

signaling increases VEGF and bFGF levels, which play a crucial role in the vascularization of the material. VEGF promotes blood vessel development by inducing endothelial cell proliferation and participates in bone development via pro-angiogenesis. Furthermore, it is involved in bone formation and metabolism as a paracrine factor. BFGF promotes the proliferation and differentiation of as well as protein synthesis in its own and surrounding cells in autocrine and paracrine ways (Hu and Olsen, 2016). Therefore, Sr-HA with pro-vascularization function can be used as a potential material, providing a new idea to solve the problem associated with vascularization in bone tissue engineering.

The role of Sr and Sr-HA in vascularization

It has been reported that bioactive ions released by some bone repair materials have an important role in angiogenesis during bone regeneration. We also found that both Sr and Sr-doped materials have an effect on angiogenesis in bone defects or osteoporotic bone regeneration. Mao *et al.,* (Mao *et al*., 2017) investigated the role of Sr ions, as well as other ions, in bioceramic materials, and showed that Sr ions enhance angiogenesis and inhibit osteoblast formation. Liu *et al.,* (Liu *et al*., 2021) selected strontium-substituted calcium silicate (Sr-CS) in their study and systematically investigated the biological functions of BMSCs-derived exosomes after Sr-CS stimulation. The results showed that Sr-CS significantly promoted *in vitro* angiogenesis of human umbilical vein endothelial cells. HA has been found to be biologically active and biocompatible under both *in vitro* and *in vivo* conditions, which is an advantageous material for bone repair (Ramesh *et al*., 2018). Thus, investigating the role of HA in vascularization and related mechanisms is essential. Sr addition into HA can improve the osteogenic capability of HA. With the excellent biocompatibility and osteoconductivity of HA, whereas Sr exhibits osteoinductive properties (Yedekçi *et al*., 2021); therefore, doping Sr greatly improves the osteoinductive characteristics of Sr-HA. The early development of several blood vessels at the implantation site of Sr-doped biological tissue engineering materials increases local nutrient supply and ion exchange, facilitates material degradation, and draws osteoblasts from the blood circulation to initiate osteogenesis. Cui *et al.,* (Cui *et al*., 2022) prepared a novel biomimetic bone scaffold containing decellularized small intestinal submucosal matrix (SIS-ECM) and

TABLE 2

STUDIES ON THE OSTEOGENIC EFFECTS OF STRONTIUM-DOPED HYDROXYAPATITE (SR-HA)

Advances on Sr-HA	Effects on Osteogenesis	Reference
Biomimetic mineralized Sr-HA bone defect repair on porous polylactic acid scaffolds	Micro-computed tomography (micro-CT) results revealed that Sr-HA /PLLA porous scaffolds could form more new bone tissues.	(Ge *et al*., 2018)
Application of Sr-HA bioactive bone cement in hip arthroplasty	Sr-HA bioactive bone cement displayed good bioactivity in the goat model of improved hip arthroplasty.	(Ni *et al*., 2006)
Strontium instead of calcium sulfate hydroxyapatite scaffold promotes bone regeneration	Pre-BMSCs, Sr-CSH /HA complex extraction significantly increased cell migration, upregulated the expression of osteogenic marker genes and increased the area of mineralized nodules.	(Chang *et al*., 2020)
Sr-HA promotes osteogenesis on polypropylene fumarate nanocomposite scaffold	Sr-HA scaffold was superior to the normal groups in supporting the adhesion, proliferation, and differentiation of MC3T3-E1 cells.	(Li *et al*., 2019)
Osteogenesis of Sr-HA coating on bone ceramic surface *in vitro* and *in vivo*	The area ratio of new bone in the Sr10-TBC group (10 mol% Sr2+ in apatite coating) was significantly higher than that in the normal group.	(Li *et al*., 2017)
3D printing Sr-HA for repairing rabbit skull defects	Sr-HA had better osteogenic ability and stimulated much more new bone formation within 12 weeks.	(Luo *et al*., 2018)
Sr-HA scaffolds prepared by the SPS technique	The material effectively repaired bone defects and displayed good biodegradable properties.	(Hu *et al*., 2020)
Sr-HA-graft-Poly(γ-benzyl-l-glutamate) nanocomposite microcarriers	Controlled Sr2+ release accelerated bone formation and promoted the repair of bone nonunion.	(Gao *et al*., 2017)
Synthesis of hydroxyapatite co-doped with trace elements Si and Sr	The measurement of osteoblast adhesion and proliferation indicated that, when compared with undoped HA, the osteoblast proliferation ability of Si HA and Si + Sr HA was increased by approximately 1.3 times and 1.8 times, respectively.	(Gao *et al*., 2016)
Effect of Sr-HA coating on bone bonding of implants in low bone mass rats	This finding revealed that 20% strontium coating had the best implant bone integration performance among the tested coatings of osteoporosis rats.	(Tao *et al*., 2016)
Effect of Sr incorporation into HA on osteoblasts *in vitro*	This study revealed that the incorporation of Sr in HA ceramics enhanced osteoblast differentiation and mineralization.	(Ni *et al*., 2011)
Osteogenesis of rat mesenchymal stem cells and osteoblastic cells on Sr-doped nanohydroxyapatite-coated titanium surfaces	The Sr-HA coating prepared by electrochemical deposition significantly enhanced the adhesion, spreading, and alkaline phosphatase activity of Sr-HA.	(Jiang *et al*., 2015)
Response of osteoprotective cells to Sr-containing HA ceramics	The results revealed that the presence of Sr stimulated the differentiation of OPC1 cells and increased the expression of ALP and OPN.	(Xue *et al*., 2010)
In vivo cancellous bone reconstruction with Sr-HA bioactive bone cement	Sr-HA forms a thick osteoid layer on the surface of bone cement, osteoblasts form along the bone and guide along the surface of bone cement, reflecting the stimulation of Sr-HA.	(Wong *et al*., 2004)
A novel injectable Sr-HA bone cement	Sr-HA bone cement slightly promoted the osteoblastic differentiation of MC3T3 cells, suggesting that Sr-HA bone cement could promote its combination with the surrounding bone.	(Dai *et al*., 2021a)
Incorporation of Sr into biomimetic carbonated calcium-deficient HA-coated carbon cloth: biocompatibility with human primary osteoblasts	The materials displayed a strong affinity with human primary osteoblasts, the incorporation of Sr in the carbonated calcium-deficient HA phase structure had a beneficial role in cell proliferation.	(Olivier *et al*., 2020)
Sr substitution for HA promotes the maturation of human osteoblasts	Qualitative evaluation using primary human osteoblasts exposed to Sr-HA for 28 days showed that, compared with HA, the presence of Sr directly promoted osteoblasts to mature into osteoblasts *in vitro*.	(Stipniece *et al*., 2021)
Synthesis and characterization of Sr-HA nanoparticles for bone regeneration	Sr-HA nanoparticles may be used to transport Sr to bone tissues and promote its regeneration.	(Frasnelli *et al*., 2017)
Sr sintered calcium sulfate bone graft promotes osteogenesis in a rat femoral defect model	The concentration of Sr2+ below 10-4M had a positive effect on the osteoblastic differentiation of MC3T3E1 cells.	(Ming-Kai *et al*., 2022)

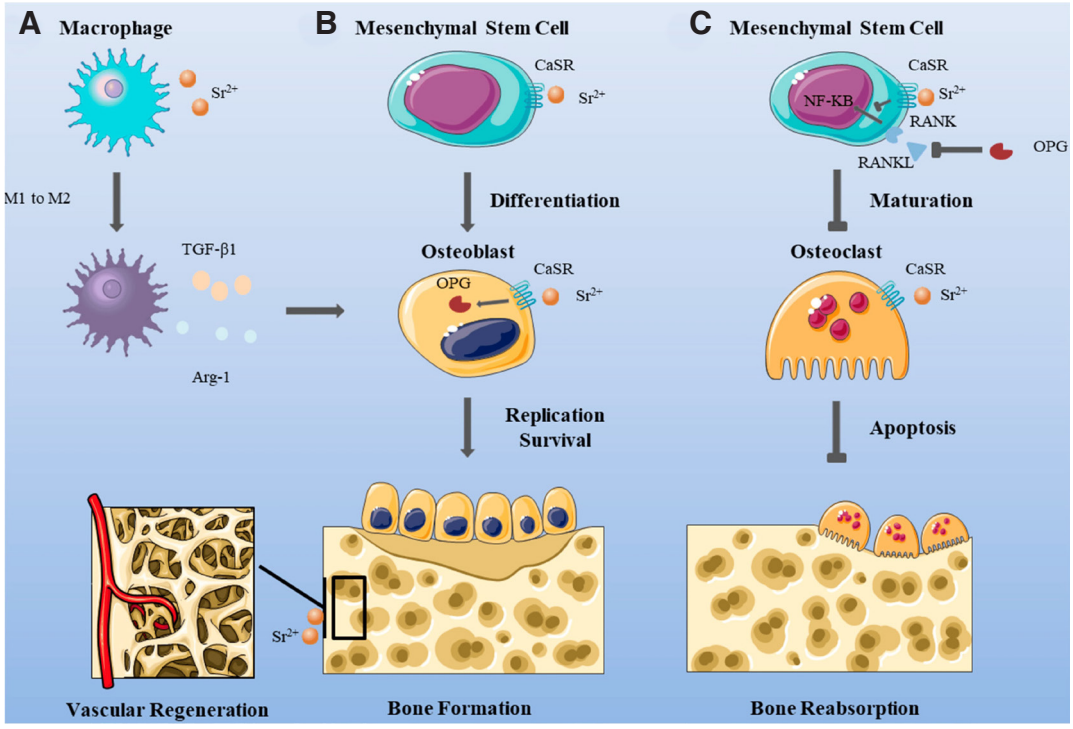

Fig. 7. Strontium (Sr) induces macrophages to differentiate toward M2 instead of M1, which benefits the promotion of osteoblast proliferation; Sr also raises early vascular regeneration. (A) Sr induces macrophage differentiation towards M2 not M1, which is conducive to promoting osteoblast proliferation, and Sr also promotes early vascular regeneration. **(B)** Sr promotes the differentiation of mesenchymal stem cell and the proliferation of osteoblasts, which is conducive to bone formation. **(C)** Sr can inhibit the differentiation of mesenchymal stem cell and the proliferation of osteoclasts, thus reducing bone resorption. Source: Liu *et al*., 2023. Licensed under CC BY 4.0.

Sr2+/Fe3+ co-doped hydroxyapatite (SrFeHA), and a series of *in vitro* and *in vivo* experiments were performed to reveal that the composites had sufficiently strong vasculogenic properties, and these positive results confirmed that the incorporation of Sr-HA enhanced the angiogenic effect. The incorporation of 10 mol% of Sr-HA significantly promoted angiogenesis by promoting cell proliferation, migration and angiogenic differentiation.

It was found that granular Sr-HA was effective in promoting the formation of bone tissue and blood vessels. Bai *et al.,* (Bai *et al*., 2018) deposited Sr-HA nanostructures onto the titanium foil surface, changed its morphology (from granular to short rods) by adjusting heating time, and verified its osteogenic and angiogenic effects *in vitro* and in vivo. The results showed that Sr in the 1% Sr-doped Ca polyphosphate material degradation solution remarkably promoted the secretion of the vascularization factor matrix metalloproteinase 2, a Zn-containing protease that degrades extracellular matrix components and the basement membrane and is a crucial pro-angiogenic factor that strongly promotes the outgrowth of new capillaries to form vascular networks. In terms of osteogenesis and angiogenesis can be found, Sr-HA is full of great potential in bone repair materials and may become a promising alternative for new bone tissue engineering.

Conclusions

Herein, we systematically review Sr-HA preparation and provide insights into the effects of implanted Sr-HA and Sr on osteogenesis and angiogenesis. Among the four preparation methods mentioned, the chemical precipitation method yields more efficient and stable materials; thus, it is the most widely used method. Additionally, Sr promotes osteogenesis by inhibiting osteoclast differentiation and promoting osteoblast differentiation. Hence, a better osteogenic effect can be achieved by adding Sr to HA. The formation and reconstruction of blood vessels play a vital role in bone healing. Sr-HA increases VEGF and BFGF levels noticeably; thus, Sr-HA can be used as a potent pro-angiogenic material. However, the involvement of inflammation and angiogenesis in bone healing leads to the complex mechanism of Sr-HA-mediated osteogenesis. Therefore, the specific osteogenic mechanism of Sr-HA needs further elucidation to maximize the effect of HA and Sr on osteogenesis.

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