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The Roles of Strontium Ions in Regenerative Dentistry: Cells Interaction, Mechanism of Action, and Future Perspective

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ABSTRACT

Strontium (Sr) ions play as a key role in the regeneration of hard tissues such as bones and teeth. Regarding the role of Sr ions in the human body, many studies have been conducted, which may be applied to hard tissue regeneration and regeneration in dentistry. In the mechanism of bone remodelling, Sr ions have a dual effect on bone metabolism: inhibit bone absorption and stimulate bone formation. From the chemical point of view, substitution of Calcium (Ca) ions with Sr in the hydroxyapatite (HA) structure will decrease its crystallinity, thus influence bone resorption process. In dentistry, diffusion of additional Sr ions from dental restorative material has also been known to have an important impact on permanent dentin in the body. In this study, 191 titles from MEDLINE (PubMed) databases and 15 titles from the Scopus database were screened to meet the relevant topic of the study. From the included studies, it was confirmed that Sr ions seem to enhance the bone quality around the implant and the osseointegration of the implant. The unique dual mode of action of Sr ion has two possible mechanisms. They balance bone renewal by activating Ca (or other cations) sensing receptors and increasing the expression of osteoprotegerin (OPG) and reducing the expression of RANK ligand by osteoblast. In various compromised cases such as patient with osteoporosis, Sr-substituted scaffold can be an option when regenerative dental therapy is needed. Further investigation is needed to fully understand the role of Sr ions in regenerative pathways and its cellular mechanism to result better choices in regenerative therapy.

Keywords
Strontium, bone remodeling, bone metabolism, cell interaction, regenerative dentistry, scaffold

Introduction

Bone remodeling is a process in which the local removal (absorption) of old bone and the replacement of new bone tissue occurs continuously. Bone remodeling is regulated both by systemic hormonal and local factors. The success of bone remodeling is determined by many factors, one of them is the supply of functional ions, such as strontium (Sr) ions. Strontium (Sr) ion is shown to increase elastic modulus, improve bone tissue quality, and decrease fracture risk

which is related to the bone microarchitecture in the biomechanical properties of bones [1]. Recently, Sr ions are also found to be an excellent factor to prevent bone fracture by stimulating osteogenesis through inducing regeneration and differentiating cells into osteoblast [2-3].

According to Kanjevac et al. [4] the diffusion of supplemented ions (such as calcium, strontium, and magnesium) from dental restorative material GIC (Glass Ionomer Cement) has an important effect on permanent teeth dentin in vivo. Since GIC can release and replenish fluoride from its surrounding environment (such as tooth structure, saliva, and dental plaque fluid) and exchange strontium with calcium, GIC can be considered as a reservoir of hydroxyapatite constituents, such as fluoride, calcium, strontium, and phosphate [5-6]. According to Brown [7] and Coulombe et al. [8], Sr can activate calcium sensing receptor (CaSR) in several cell types which produces inositol triphosphate (IP3) and mitogen activated protein kinase (MAPK). Strontium is also found to be able to induce cyclooxygenase-2 expression and prostaglandin E2 (PGE2) by activating extracellular signal regulated kinases (ERK) involved in bone formation in osteoblasts [9] wherein Sr ions affect bone cell in vitro by bone resorption inhibition and bone formation promotion [10]. Based on the in vitro and in vivo studies, a combination of Sr with hydroxyapatite (HA) can promote osteoblast proliferation and regulate osteoclastogenesis within normal cells and osteopenic by increasing alkaline phosphatase (ALP) activities [11-13].

On the other hands, bone tissue continuously remodels through the actions of osteoblasts and osteoclasts [14]. Bone remodeling can adjust bone structure to meet the needs of mechanical changes, help repair micro-damages, and remove old tissue [15]. The cell generates growth factors, cytokines, and non-collagenous protein that regulate bone remodeling [16]. In this mechanism, Sr ions have dual effects in bone metabolism: to inhibit bone resorption and stimulate bone formation [17]. Level of Sr ions in teeth is correlated to its concentration in drinking water and influenced DMF (decayed, missing, and filled teeth) values in low fluoridated areas [6, 18]. Strontium and fluorine are present in glass ionomer cement (GIC), which is used for the bases or restorations of primary and permanent teeth [6].

Strontium ions accumulated in the bone tissue by 99% and is located in the body in which Sr and Ca ions have similar charges to size ratio. Therefore, Sr ions could potentially substitute Ca ion in apatite [19]. The highest 10-12% Sr ions which are found in biological apatite becomes the current standard [20-21]. Strontium in bone metabolism can stimulate osteoblast proliferation during the deceleration of osteoclast activity and increase bone mineral density. Strontium is also found to be able to act as medicines [22-24] and biomaterial [13, 25] in the osteoporosis treatment development.

Based on the previous studies, Sr ions are known to play a key role in the regeneration of hard tissues such as bones and teeth. Regarding the role of Sr ions in the human body, many studies have been conducted, which may be applied to hard tissue regeneration and regenerative dentistry. This review is significant to understand the current status of Sr ions function in growing and healing bone cell (osteoblast), increasing microarchitecture/ mechanical strength of the bone, and also accelerating bone regeneration (e.g., bone remodeling) in bone grafting techniques. The understanding on the roles of Sr ions in bone remodeling and its mechanism of

action can be a platform in developing prospective regenerative treatment in dentistry and medicine.

Materials and Methods

Criteria in considering studies for the review.

This study is a brief or concise review on the role of Sr ions in regenerative dentistry. Although it is not a systematic review or meta-analysis, the study was carried out with the PRISMA (the preferred reporting item for systematic reviews and meta-analysis) as described by Moher et al. [26] and Cochrane's manual on systematic review of interventions by Higgins and Green [27] as a framework.

The effectivity and toxicity of accurate concentration levels of Sr ions produced as a bone graft in the form of scaffolds or medicines were reviewed whether it has the potential in the regeneration of tissues related to dentistry. The retrieval of data information focuses on (1) mechanism, (2) type of treatment with Sr ions, (3) interpretation of research results, and (4) side effect of a certain treatment. If the study failed to report one or more among four of the intended information, the data from the paper will still be considered and acknowledged in the review.

Search strategy for identification of studies.

The MEDLINE (Medical Literature Analysis and Retrieval System Online, via PubMed) and Scopus databases have been searched until January 2021 for studies evaluating Sr ions as biomaterial composites in the form of scaffolds in regenerating bone tissue in medicine and dentistry. The search strategy is limited to English publications that use a combination of keywords such as strontium, regeneration in dentistry, implantation, biomaterial or biocompatibility, and bone graft or scaffold. Systematic reviews and reviews were immediately included when it is relevant, as well as case reports which indicated prior safety preclinical experiments. A manual search was performed based on the reference list of the selected papers. The electronic database of the following journals was searched manually, which are important for this review, for example, Journal of Dentistry, Materials Science and Engineering C, Scientific Reports, Acta Biomaterialia, International Journal of Molecular Sciences, Calcified Tissue International, Osteoporosis International, Oral Pharmacology and Oral Medicine. Further, the bibliographic references of included studies were also sought for possible related studies. The authors independently filter the title, abstract, and full text of the search results. When there is a disagreement, the authors discuss the research to reach a consensus.

Study selection and data extraction.

The researchers conducted data extraction and validity evaluation of the studies that met the inclusion criteria. According to the content of each research report, data for regenerative dental treatments are extracted (for examples implants, bone grafts, biocompatibility, cytotoxicity, biomechanical). According to Hooijmans et al. [28] the quality assessment of studies involving animals is based on the risk of the SYRCLE's bias tool (no summary scores for the studies were

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included). Reviewers independently assessed the quality of the study. When there were disagreements on the evaluation data, consensus discussions were held.

Results and Discussions

Search results.

The applied search term was “strontium” [MeSH Terms] OR “strontium” [All Fields] AND "regenerability"[All Fields] OR "regenerable"[All Fields] OR "regenerant"[All Fields] OR "regenerants"[All Fields] OR "regenerate"[All Fields] OR "regenerated"[All Fields] OR "regenerates"[All Fields] OR "regenerating"[All Fields] OR "regeneration"[MeSH Terms] OR "regeneration"[All Fields] OR "regenerations"[All Fields] AND "biocompatability"[All Fields] OR "biocompatibilities"[All Fields] OR "biocompatibility"[All Fields] OR "biocompatible"[All Fields] in PubMed for the articles written in English published up to January 31, 2021. Preliminary search for data publication consisted of 183 titles from MEDLINE (PubMed) databases and 15 titles from the Scopus database. Table 1 shows number of articles found when necessary queries were included among 183 MEDLINE (PubMed) search results.

Table 1. Search results and added queries.

Search Details	Added Query	Number of articles
((Strontium) AND (Regeneration)) AND(Biocompatibility) Filters: English, from 1000/1/1 - 2021/1/31	None	183
(((Strontium) AND (Regeneration)) AND (Biocompatibility) AND ((1000/1/1:2021/1/31[pdat]) AND (english[Filter]))) AND (Osteoporosis) Filters: English, from 1000/1/1 - 2021/1/31	Osteoporosis	29
(((Strontium) AND (Regeneration)) AND (Biocompatibility) AND ((1000/1/1:2021/1/31[pdat]) AND (english[Filter]))) AND (Dentistry) Filters: English, from 1000/1/1 - 2021/1/31	Dentistry	36
(((Strontium) AND (Regeneration)) AND (Biocompatibility) AND ((1000/1/1:2021/1/31[pdat]) AND (english[Filter]))) AND (Scaffold) Filters: English, from 1000/1/1 - 2021/1/31	Scaffold	64

Based on that, it was considered that the search results covered all necessary information for this review. In this study, search terms “strontium” AND “bone” AND “systematic review” were also

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used to browse the articles in English published in PubMed up to January 31, 2021 to screen the ones conducted as meta- analysis or systematic reviews. There were 38 articles resulted. After title screening, authors decided to only use 8 articles among them, wherein 1 of 8 [29] was also included in the previous 183 MEDLINE (PubMed). The remaining 30 articles were excluded because they were not relevant to this study. Thus, in total there were 206 articles for further analysis in this study (Table 2).

Table 2. Number of search results for further article analysis

Sources	Query	Record of identified articles	Number of articles for further analysis
MEDLINE (PubMed) – Group 1	Strontium, regeneration, biocompatibility	183	183
Scopus – Group 2	Strontium, regeneration, biocompatibility	15	15
MEDLINE (PubMed) – Group 3	Strontium, bone, systematic review		
	- Included in Group 1, relevant to the study	1	0 Remarks: Has been included in Group 1 for further analysis
	- Not included in Group 1, relevant to the study	7	7
	- Included in Group 1, not relevant to the study	0	0
	- Not included in Group 1, not relevant to the study	30	0
Total Number of Articles:		**236**	**205**

After the screening process, there were 150 titles that were relevant to the main topic. Once the initial scanning based on abstracts and keywords was done, 28 publications (24 from PubMed and 4 from Scopus) were found to potentially serve as a criterion in developing topics. A certain publication that is deemed to be not relevant to the main topics after being reviewed was excluded. From the selection of the text, the main indicator in excluding the publications is the methodology and the function of Sr ions in dentistry.

As a result, authors indicated that the function and usefulness of Sr ions in dentistry are relevant for this journal review. There were no additional publications found from bibliographic reference and hand searching as additional data information in the journal. A lot of information related to

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strontium application was found but they have not discussed further in detail. The studies selection process is depicted in the flowchart as shown in Figure 1.

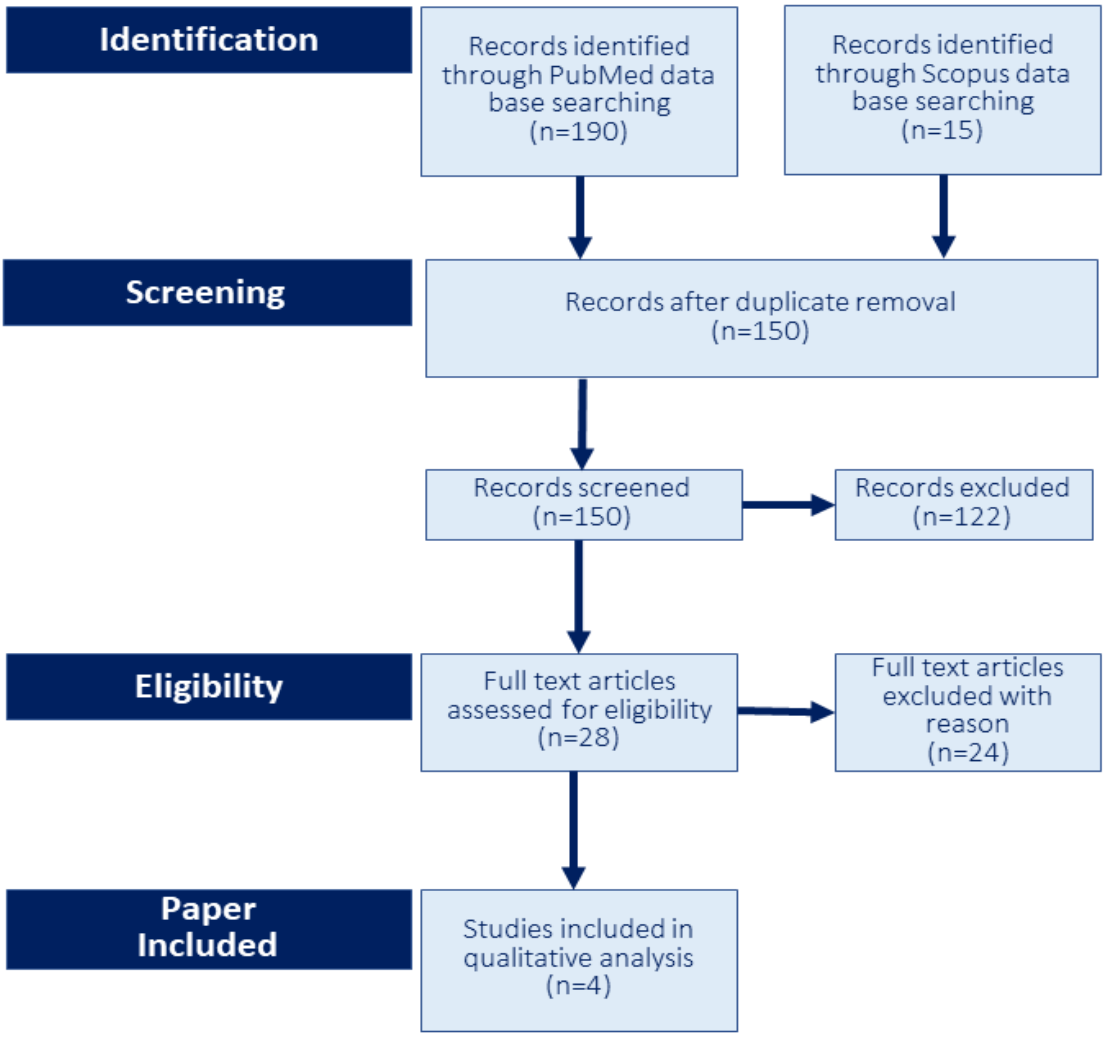

Figure 1. Studies selection process referred to PRISMA flow diagram.

It has been notified that there were 4 papers included for further reading in this literature review (Table 3). In 2018 Scardueli et al. [30] conducted a systematic review of strontium ranelate (SRAN) in animals. The completed systematic review obtained 578 titles in the MEDLINE (PubMed) database and 152 titles in the Scopus database. After abstract and keyword screening, the remaining 37 publications (31 from PubMed and 6 from Scopus) may meet the inclusion criteria. A full-text reading to the selected 37 publications excluded publications based on Sr local delivery rather than systemic usage. In the final, there were only 5 publications for further qualitative analysis. According to the results of the systematic review, it was found that Sr ions seem to enhance the bone quality around the implant and the osseointegration of the implant. Scardueli et al. (2018) also proposed the focus of further studies which includes dose, administration starting point, duration, and potential of risks- benefits on the use of SRAN.

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Table 3. Relevant studies included in the review.

Studies included (Authors and Year)	Method	Results	Relevance	Recommendations
Scardueli et al., 2018 [30]	A systematic review on the systemic administration of Sr ranelate (SRAN) in animal	Among 578 yielded studies, in the final, there were only 5 publications for further qualitative analysis. It was found that Sr ions seem to enhance peri-implant bone quality and implant osseointegration.	Enhancement of peri-implant bone quality and osseointegration by Sr ions.	Further studies which includes dose, administration starting point, duration, and potential of risks-benefits on the use of SRAN are recommended.
Hamdy, 2009 [31]	A review on the role of SRAN to improve bone microarchitecture in osteoporosis	It was proposed from the study that there are 2 putative mechanism of the unique dual mode of action of SRAN which rebalance bone turnover in favor of bone formation by activation of Ca (or other cations) sensing receptor and increase of osteoprotegerin (OPG) expression coupled with decrease RANK ligand expression by osteoblast.	Mechanism of action of Sr ions in improving bone biomechanical properties.	Investigation on the use of other anti- resorptive and anabolic agents is needed.
Ehret et al., 2017 [32]	An in vivo study on the effect of Sr-doped hydroxyapatite polysaccharide materials to the development of ectopic bone formation.	It was found that Sr ions supported osteoblastic differentiation, osteopontin (a marker which involves in the mineralization process) activation, osteoid tissue induction, and blood vessels formation even in non- osseous environment. It was found the absence of cytotoxicity of the incorporated Sr.	Enhancement of bone formation by Sr- doped scaffold in non- osseous environment.	Further preclinical studies to demonstrate the safety and efficacy of the composite materials in large bone defects are needed.
Tovani et al., 2017 [33]	In vitro study on the formation of stable strontium-rich amorphous calcium phosphate and its possible effects on bone mineral.	A bioinspired pathway on the model of Sr-ACP phase formation and how it affects bone was observed. A 10 at% of Sr^{2+}, as a physiological Sr ions limit incorporated in bone, can be incorporated into HA without phase segregation.	Pathway on the formation of Sr-ACP phase and its affect to bone.	Further investigations to understand the stability of the Sr-ACP phase on 3D (3-dimension) collagen matrices to mimic bone environment are recommended.

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A review entitled "SRAN improves bone microarchitecture in osteoporosis" was also found relevant to this study [31]. The study started from the basic consideration, that is, the destruction of bone reconstruction will lead to bone loss, microstructure damage, and increased risk of fractures, which has been known to be the profound problem in osteoporosis. Therefore, if antiresorptive and anabolic agents which can suppress the bone remodeling disruption are available, that will absolutely resolve the osteoporotic problems.

Moreover, the review [31] proposed two speculative mechanisms for SRAN's unique dual mode of action. The mechanism balances bone renewal by activating Ca (or other cations) sensing receptors and increasing osteoprotegerin (OPG) expression and reducing RANK ligands expression by osteoblast. The review conducted in the study [31] was based on extensive in vitro and in vivo preclinical studies. SRAN can restore the imbalance between bone resorption and formation, which is beneficial to the treatment of osteoporosis and is a novel and unique methods. Bone formation and its beneficial effect on bone microstructure, thereby improving the biomechanical properties of bone.

An experimental study by Ehret et al. was also found to meet the inclusion criteria [32]. In the study, a test on the effect of Sr-doped hydroxyapatite polysaccharide materials on the development of ectopic bone formation was conducted. This research is an important point to show the roles of Sr ions supplementation to bone formation, even in non- osseous environment. The study also showed that no matter how much Sr is incorporated, the composite material doped with Sr has no cytotoxicity. It was also found that Sr ions supported osteoblastic differentiation, osteopontin (a marker which involves in the mineralization process) activation, induction of osteoid tissue and formation of blood vessels. From their study, Ehret and co- workers proposed further preclinical studies to prove the safety and effectiveness of the composite materials in large bone defects [32].

The fourth study included in this review was a study by Tovani et al. [33]. The study is more basic compared to others 2 included studies. Tovani and co-workers reported Sr-ACP as a new stable formation amorphous calcium phosphate (ACP) phase with Sr^{2+}-rich. They showed a bioinspired pathway on the model of Sr-ACP phase formation and how it affects bone. They also described that as the physiological limit of Sr ions incorporated in bone, Sr^{2+} of no more than at 10% can be incorporated into HA without phase separation. It was also projected from the study that the comprehensive model found in the study can be used to describe the role of functionally added ions and biologically interesting molecules in the bone mineralization pathway. Further research has been proposed to understand the stability of the Sr-ACP phase on 3D (3-dimension) collagen matrix to mimic bone environment.

Functions of Sr ions in osteoblast and osteclast

In general, Sr is defined as a trace mineral in the human body (accounting for 0.00044% of body weight) which is classified in the form of strontium hydroxide or $Sr(OH)_2$ in animal tissue [34]. Strontium is a trace metal element where approximately 98% of Sr in human body is located in bone tissue. On bone regeneration applications, Sr has similarities to calcium in structural, physical, and chemical properties [35]. Strontium is frequently used to enrich biological

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materials, such as calcium phosphate, bioactive glasses, bone cement, and metal implants. The presence of Sr^{2+} in the structure of biomaterials stimulates proliferation and osteogenic differentiation of osteoblasts, and inhibits the activity of osteoclasts in vitro [35]. Table 4 shows the application of Sr ions in clinics due to its affinity to bone [22, 36-45].

Table 4. Strontium application due to its affinity to bone

Reference	Application	Year of Application
Altman and Lee, 1996 [36]	The use of SR-89 to treat bone pain related to metastatic bone cancer. Strontium functions as adjunctive element to chemo, radiation, and hormonal therapy.	1940
Shorr and Carter, 1952 [37]	The use of Sr- lactate (stable non-radioactive Sr) to remineralize the skeleton taken along with Ca supplement.	1952
Rosenthall, 1963 [38]	The use of Sr-85 in bone lesions imaging of patients with bone cancer.	1965
Morohashi et al., 1994 [39]; Morohashi et al., 1995 [40]; Marie et al., 1985 [41]; Marie and Hott, 1986 [42]	The use of Sr as an anti-osteoporotic agent after estrogen deficiency in ovariectomixed animals. This study led to clinical testing.	1985
Reginster et al., 2002 [22]; Meunier et al., 2002 [43]	The pharmacological use of SrRan or SRAN which is composed of two stable Sr atoms and ranelic acid was approved to treat osteoporosis.	Phase 2 Clinical Trials in 2002
Reginster et al., 2005 [44]; Meunier et al., 2004 [45]	The use of SRAN for the treatment of spinal and peripheral osteoporosis.	Phase 3 Clinical Trial in 2004

Strontium-hydroxyapatite (Sr-HA) hybrid promotes the proliferation of normal cells and osteoblasts in osteoporosis and down-regulates osteoclast production by increasing ALP activity in vitro and in vivo studies [11-13]. As it has been noted that ALP is responsible to convert organic phosphate into the inorganic one that will allow the reaction of inorganic phosphate with calcium ions to form mineralized matrix [46]. Taken from another study, a hybrid of bacterial cellulose and hydroxyapatite can be functioned as Sr ions delivery. Luz and co-authors [47] conducted a study on the chemical structure, morphology, porosity, surface composition, and swelling degree of bacterial cellulose (BC) and hydroxyapatite (HA), including their adsorption

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and desorption capabilities through two different ways. For the first approach on the characterization of the synthesis of hybrid compounds, the hybrid compounds are generated, and then immersed in a solution of strontium nitrate ($Sr(NO_3)_2$) to promote the exchange of calcium ions with Sr ions. For the second approach, Sr ions were immediately penetrated the BC matrix, causing strontium apatite to precipitate on the BC fibre [47].

In others study, Huang et al. [34] evaluated the differentiation of human dental pulp stem cells (hDPSCs) in the condition with or without exogenous Sr ions added to the fusion culture at the concentration of chloride $SrCl_2.6H_2O$. It was found that addition of Sr ions modulates the DSPP (dentine sialo phosphoprotein) and DMP-1 (dentine matrix protein 1) secretion via Ca-SR (calcium sensing receptor) using similar pathway with osteoblast differentiation. From the study, it was projected, and further investigations were proposed that if Sr ions are added to dental applications, Sr ions may increase hDPSCs metabolic and ALP activities which could have the potential to induce dentine- like matrix formation [34].

When bone precursor cells attach and proliferate, ten percent of Sr ions are found to be very good without eliminating the effect on the formation and mineralization of extracellular matrix or ECM [48]. Sr-HA can stimulate the osseointegration of implants and has the potential to be used as dental alternative materials, fillers, drug carriers, and hard tissue scaffolds with lower Sr concentration of nanoparticles. These elements have been evaluated for their physical and chemical properties as well as their cytocompatibility [49]. The implantation of HA materials containing Sr can promote bone repair in normal and ovariectomized animals, and enhance fracture healing [2, 49-50]. Recently, a study by Luo et al. [51] showed that after ectopic implantation combined with rhBMP-2, the possible synergistic effect of rhBMP-2 and Sr on bone formation can enhance the bone formation of Sr-containing apatite composites.

Several in vitro experiments on the effect of Sr in bones with respect to dose administered have been conducted. Biomechanical and histomorphometric test on a skeleton proves that Sr has no toxicity effect on bone cells and mineralization at a dose of less than 1% in a diet [41-42, 52-53]. Based on the literature, a low dose of Sr in the body is below 4 mmol/kg/day. High doses of Sr might induce skeletal anomalies, rickets, and defective mineralization of bone [54-66]. In rats, high doses of Sr reduce the activity of 25-hydroxyvitamin D3α-hydroxylase [54-56]. Experiments have shown that low doses of Sr can inhibit bone absorption and stimulate bone formation, thereby improving the bone quality of normal animals [57]. Table 5 summarizes the in vitro and in vivo effects of SRAN ions or Sr ions in the form of Sr or Sr on bone.

Mechanism of Sr action in bone remodeling

Under normal circumstances, bone remodeling is used to adjust bone structure to meet changing mechanical requirements, and to help repair micro-damages and remove old tissue [15]. In the tissue, cell generates growth factors, cytokines, and non-collagen protein that regulate bone remodeling [58]. Some studies show that bones are complex tissues that undergo a continuous remodeling process through the action of osteoblasts and osteoclasts in which the application of Sr ions into a synthetic microenvironment such as Sr-HA has a role related to bone remodeling [14, 47, 59].

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Table 5. Summary of the in vitro and in vivo effects of Sr ions or SRAN to bone

In Vitro	In Vivo
• Increasing osteogenic differentiation of BMSC • Stimulating osteoblastic differentiation of hMSCs • Increasing osteoblastic proliferation and differentiation • Reduction of osteoblast apoptosis • Decrease osteoclast differentiation/ activation • Increase osteoclast apoptosis	• Increase bone mass and density • Improve bone mechanical properties and strength • Reduction of new vertebral, nonvertebral, and hip fractures

In osteoporosis case, bone remodeling process is disrupted, leading to increase fracture risks. In the case of osteoporosis, Sr ions have a dual mode of action, which can support bone formation by balancing bone renewal, while activating calcium or other cation-sensing receptors, and increasing the expression of osteoprotegerin (OPG), and by reducing RANK ligand expression by the osteoblasts [31]. Micro-CT analysis of bone biopsies of patients undergoing Sr treatment showed that the intrinsic quality of the bone was improved, which was characterized by an increase in the number of trabeculae, a decrease in trabecular separation, a decrease in structural model index, and an increase in cortical thickness [1, 31].

Sr can not only improve the mechanical properties of minerals, but it can also regulate bioactive properties, such as osteo inductivity [60]. In vitro Sr-doped hydroxyapatite (HA) facilitates the proliferation and differentiation of osteoblasts [61-64]. At low doses, Sr can stimulate bone mineralization while it has detrimental effects on high doses [50, 65-66]. Based on in vitro experiments on the low and high concentration of Sr (8% and 50%), there is no cytotoxicity in which cell proliferates in MSCs during 7 days of culture [32]. Some literatures explain that Sr contained materials stimulates the MSCs or other osteoblast lines differentiation [11, 63]. A matrix of Sr doped- HA can differentiate MSCs and osteoblast, and express specific genes (Runx2 and OPN) as well. The implantation of Sr-HA can promote bone healing by stimulating bone fracture healing and inducing ectopic bone formation [51].

On the other study, the presence of Sr ions can enhance the proliferation and osteogenic cell and inhibit osteoclast activity in vitro. Glass replaced with 5% Sr can promote the ALP activity and mineralization of MC3T3 cells in vitro, and incubation of 10 mM SRAN together can accelerate the mineralization of osteoblasts in the co-culture model and stimulate osteoclast differentiation [3, 67]. Strontium can also stimulate the dual regulation mode of bone formation by increasing the production of osteoprotegerin (OPG) and down-regulating the expression of RANKL in osteoblasts through calcium-sensing receptors [8, 68-69]. According to the study by Pilmane et al. [70] OPG can be used as a decoy receptor for RANKL, which can inhibit the differentiation of osteoclasts induced by RANKL, which can weaken the differentiation of osteoclasts and weaken bone resorption. Figure 2 shows the mechanism of Sr ion in the bone remodelling process.

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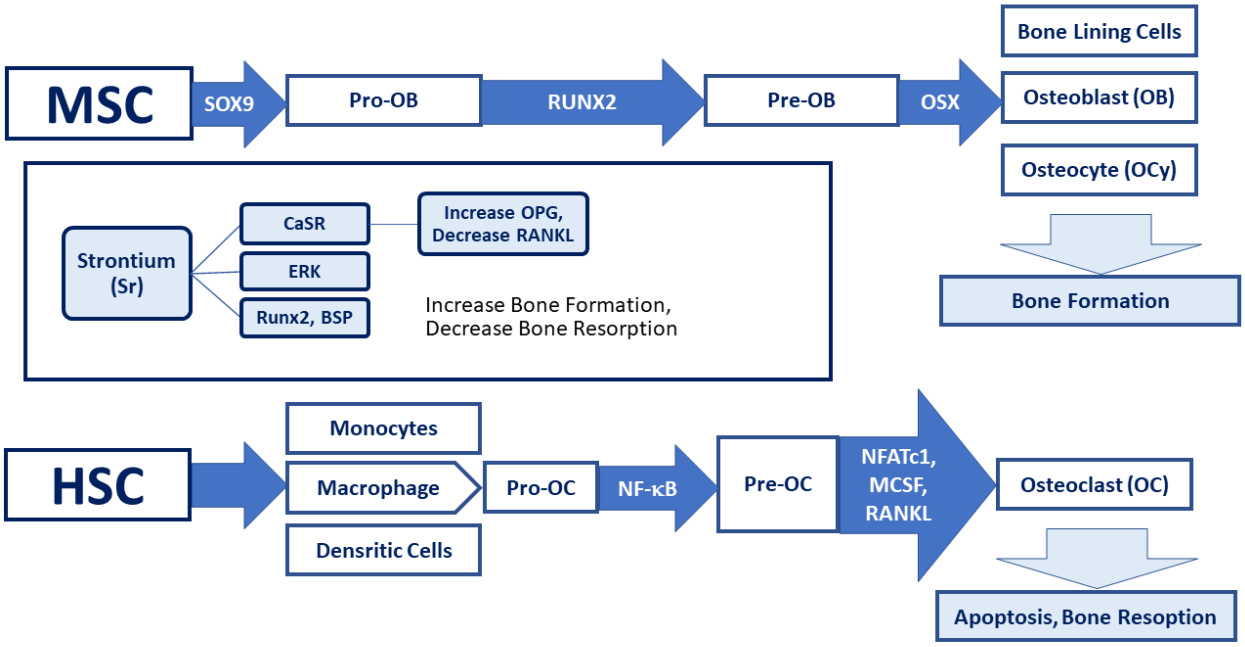

Figure 2. Strontium ions can activate CaSR that will activate osteoblastic replication and OPG production. OPG suppress differentiation of osteoclast by inhibition of NF-κB and RANKL.

Strontium is transported in the blood by binding to serum proteins that usually carry calcium. Serum proteins usually carry calcium and compete with calcium for absorption in the intestine and renal tubules [57]. Toxic Sr doses can cause normal Ca homeostasis disorders, such as hypocalcaemia and impaired bone mineralization [39-40]. A protein receptor located in bone cells, called CaSR (calcium sensitive receptor), plays a key role in maintaining calcium homeostasis. This protein receptor allows cells in the parathyroid glands and renal tubules to sense extracellular calcium and regulates the secretion of parathyroid hormone (PTH) and the processing of calcium by the kidneys [71]. The extracellular calcium concentration indirectly affects bone remodeling by changing the levels of PTH and vitamin D3. Recent findings indicate that bone cells can also directly sense calcium levels and respond accordingly. In vitro reports provide evidence that osteoblasts, osteoclast precursors and mature osteoclasts express parathyroid CaSR homologs [72-74]. In these cell types, CaSR has been shown to respond to Ca^{2+} binding and other divalent cations (such as Sr^{2+}) to control key cell functions, such as cell growth, differentiation, and apoptosis [75-76].

According to in vivo studies, Sr/SRAN has a positive effect on bones. Despite the positive effects of Sr ions to bone, the triggered mechanism is not completely known. This mechanism has been being studied intensively by biomaterial scientists, to provide new combination of Sr and other materials. The first possible event determined by biomaterials scientists is that CaSR is activated by extracellular divalent cations such as Ca and Sr (which have lower affinity than Ca), leading to the production of inositol-1,4,5-triphosphate (IP3) and mitogen-activated protein kinase (MAPK) signalling. Through this mechanism, Sr can activate osteoblast replication through CaSR.

Although it has been reported that in this mechanism, Sr ions can activate the mitogen-mediated protein kinase (MAPK) pathway and regulate calcium-dependent cell signaling pathways through CaSR, which is a common way for osteoblasts to increase cell activity physiological receptors, but the exact mechanisms behind these functions have not been well studied or understood [70].

The second mechanism is related to the ability of Sr to activate the 1/2 phosphorylation of extracellular signal-regulated kinase (ERK). In addition to CaSR, another receptor can also mediate the effect of Sr on osteoblast replication. This mechanism suggests that Sr induces the expression of cyclooxygenase (COX)-2 and prostaglandin E2 (PGE2) by activating the ERK signaling pathway [9, 70, 77-79]. The third hypothetical mechanism is that Sr in osteoblasts that can inhibit bone resorptions, increase cellular activities of osteoprotegerin (OPG) and reduce the receptor activator of nuclear factor kappa B ligand (RANKL) expression [80]. OPG is the decoy receptor of RANK. When OPG is activated, it inhibits the activity of osteoclastogenesis by inhibiting the production of osteoclasts by RANKL [80-81]. Therefore, the increased expression of OPG and the inhibition of RANKL production by osteoblasts at different stages of differentiation weakened the differentiation and function of osteoclasts. Since the OPG / RANKL system plays a key role in the differentiation of osteoclasts, it is shown that the CaSR signal changes caused by Sr play a key role in mediating the regulation of the OPG/ RANKL system.

Strontium as therapy for osteoporosis

Increasing knowledge about the pathogenesis of osteoporosis has paved the way for understanding the mechanism of the beneficial effects of Sr ranelate on bones. The in vitro and in vivo preclinical evaluation of postmenopausal women and the subsequent comprehensive clinical plan have made people realize that Sr can restore the imbalance between bone resorption and formation, which is beneficial to the treatment of osteoporosis. This novel and interesting method affects the microstructure of bones, improves the biomechanics and strength properties of bones, and has proven anti-fracture effects [31].

Strontium has a promising anti- osteoporotic treatment. It shows that when incorporated into biomaterials, Sr ions can be released to the surrounding environment. If it is achieved, Sr can be used in orthopaedics, spinal surgery, and dental surgery for hard tissue regeneration [34, 82]. Strontium also serves as anti-resorptive and one formation agent [76] where it can inhibit bone resorption by 30% by reducing osteoclast activity, measured by pit assay on cell isolation of rats [83]. Although there are still doubts about the effective level of Sr concentration that inhibits the activity of bone osteoclasts in bone in vitro, a high concentration of Sr binding with HA is found on the surface of bone crystals [19].

In vitro experiment shows that Sr ions can inhibit the recruitment and activity of osteoclasts. For example, carbonic anhydrase II in bone and the αv subunit of vitronectin receptor can inhibit the differentiation of pre-osteoclasts into osteoclasts [84]. Related to osteogenic differentiation of rat calvaria from osteoblasts (such as MC3T3E1). It was found that Sr can stimulate ALP activity, a marker of osteoblast differentiation and collagen synthesis in the internal system without affecting matrix mineralization, thereby the bone formation activity of Sr in vitro was confirmed [85].

At pH 8, the effect of Sr on the proliferation of osteoblasts is even stronger, indicating a feasible new method for enhancing its activity in the treatment of osteoporosis. Strontium is also capable to induce the expression of cyclooxygenase-2 and PGE2 through the activation of an extracellular which regulates kinase in osteoblast that includes bone formation effects [9] where Sr affects bone cell by inhibits bone adsorption and promotes bone formation [10]. For adult rats, Sr can increase the bone mass/volume of the lumbar spine and femur based on histological analysis [1]. This can increase the trabecular thickness and lower trabecular separation in tibial metaphyses which indicate an improvement of bone microarchitecture, where it can enhance bone strength based on mechanical properties of the axial (lumbar spine) and appendix skeleton (humerus and femur). Moreover, elevated plasma ALP increases compatibility and bone formation activity [1].

Strontium can reduce bone resorption and increase mineralization of the surface of alveolar bone [86]. Evidence-based pharmacology shows that Sr can induce anti-resorption and in vivo bone formation effects which result to increased bone mass and bone strength of vertebrae and appendix bones. Strontium adsorbs on the crystal surface of bones, and only 1 out of 10 Ca cation in apatite crystals that is replaced by Sr in the body [11]. The analysis of HA form under Sr treatments shows no different results in the degree of mineralization in vitro [87]. High doses of Sr given to rats for two years do not show changes in the results of bone mineralization [1].

Future perspective

The combination of fluoride with strontium and alumina provides better protection for tooth structure, preventing demineralization and microbial attack. It also enhances the remineralization of restorative materials. However, when testing several known bacteria directly with a strontium chloride hexahydrate solution within a short period of six minutes, Sr itself does not exert an antibacterial effect [6, 88]. Therefore, if Sr ions is incorporated into dental restorative materials, such as in GIC, it would certainly be an advantage for restorative dental treatment.

Strontium was observed to modulate the OPG/RANKL ratio, leading to an increase in OPG secretion and a decrease in RANKL expression, which leads to the inhibition of osteoclastogenesis [70]. In another word, Sr ions were also confirmed to stimulate bone formation by increasing the production of osteoprotegerin (OPG) and downregulating the expression of RANKL in osteoblasts through CaSR [8, 68-69]. The positive effect of Sr ions for bone remodeling mechanism can be a prospective regenerative therapy if Sr ions are incorporated into regenerative scaffolds to fasten bone tissue regeneration upon oral maxillofacial surgery, promote alveolar bone regeneration caused by periodontal diseases, or enhance osseointegration in dental implantology. In various compromised cases such as patient with osteoporosis, Sr- substituted biomaterials can be an option when regenerative dental therapy is needed. Furthermore, orthodontics treatment which requires relapse prevention can also benefit by the application of Sr- containing devices.

From the chemical structural point of view and the relevance with bone graft, implantation, and scaffold development, Sr was found to reduce hydroxyapatite crystallinity. Singh et al. [89] suggested the potential of Strontium (Sr) ion in bone tissue regeneration. Ozbeck et al. [90] performed Sr ion doping on HA powder and found that the addition of Sr in HA crystals causes

substitution of calcium (Ca) ions which bind to hydroxyl (OH) by Sr. The partial substitution of Ca by Sr produces an ionic diameter of Sr - OH that is greater than that of Ca - OH which in turn results in a decrease in lattice energy and crystallinity in HA mineral. Thus, chemically the addition of Sr in bone mineral can cause changes in the structure of apatite to produce apatite with low crystallinity. This is a specific key point for the technological development related to scaffold engineering.

Conclusion

According to the studies discussed in this review, Sr ions have a great potential in dentistry, particularly in treating bone fractures, osteoporosis, and modulating bone remodeling, Many literatures present that Sr can be composited with different materials to enhance mechanical and biocompatibility. Strontium plays a dual role in bone metabolism by stimulates bone formation and inhibits bone resorption. Future study is needed to understand completely the detailed mechanism on the roles of Sr ions in bone remodeling pathways. A standardized concentration related to its use in regenerative dentistry is an open area to be investigated further. The incorporation of Sr ions into newly developed scaffolds for wound healing in dentistry, oral and maxillofacial regenerations, orthodontics treatment, dental implantology, and periodontal regeneration is awaiting to be studied to resolve problems in regenerative dentistry.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Compliance with ethical standards

This article does not contain any studies involving human participants or animals performed by any of the authors.

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