BJR

RESEARCH

In vivo and clinical application of strontium-enriched biomaterials for bone regeneration

A SYSTEMATIC REVIEW

N. Neves,
D. Linhares,
G. Costa,
C. C. Ribeiro,
M. A. Barbosa

Centro Hospitalar de São João – Orthopedic Department, Porto, Portugal

N. Neves, MD, Researcher, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Universidade do Porto, Rua Alfredo Allen, 208, 4200-135 Porto and Researcher, INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Rua Alfredo Allen, 208, 4200-135 Porto and Lecturer Faculty of Medicine, University of Porto, Surgery Department, Alameda Prof. Hernâni Monteiro, 4200-319 Porto and Orthopaedic Surgeon Centro Hospitalar de São João, Orthopedic Department, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal.

D. Linhares, MD, Orthopedic Resident, Orthopaedic Department, Centro Hospitalar de São João, Alameda Prof. Hernâni Monteiro, 4200-319 Porto and Researcher and Lecturer, MEDCIDS - Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto Portugal.

G. Costa, PhD, Professor, Faculty of Medicine, Surgery Department, University of Porto, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, and Orthopaedic Surgeon, Centro Hospitalar de São João, Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal.

C. C. Ribeiro, PhD, Researcher, Instituto de Investigação e Inovação em Saúde, Rua Alfredo Allen, 208, 4200-135 Porto, Portugal and Researcher, INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Rua Alfredo Allen, 208, 4200-135 Porto and Professor, ISEP - Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto, Rua Dr. António Bernardino de Almeida 431, 4249-015 Porto, Portugal.

M. A. Barbosa, PhD, Researcher, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Rua Alfredo Allen, 208, 4200-135 Porto and Researcher, INEB - Instituto de Engenharia Biomédica, Universidade do Porto, Rua Alfredo Allen, 208, 4200-135 Porto and Professor, ICBAS-Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal.

Correspondence should be sent to N. Neves;
email: nsmneves@gmail.com

doi: 10.1302/2046-3758.66.BJR-2016-0311.R1

Bone Joint Res 2017;6:366–375.
Received: 23 November 2016;
Accepted: 28 February 2017

Objectives

This systematic review aimed to assess the *in vivo* and clinical effect of strontium (Sr)-enriched biomaterials in bone formation and/or remodelling.

Methods

A systematic search was performed in Pubmed, followed by a two-step selection process. We included *in vivo* original studies on Sr-containing biomaterials used for bone support or regeneration, comparing at least two groups that only differ in Sr addition in the experimental group.

Results

A total of 572 references were retrieved and 27 were included. Animal models were used in 26 articles, and one article described a human study. Osteoporotic models were included in 11 papers. All articles showed similar or increased effect of Sr in bone formation and/or regeneration, in both healthy and osteoporotic models. No study found a decreased effect. Adverse effects were assessed in 17 articles, 13 on local and four on systemic adverse effects. From these, only one reported a systemic impact from Sr addition. Data on gene and/or protein expression were available from seven studies.

Conclusions

This review showed the safety and effectiveness of Sr-enriched biomaterials for stimulating bone formation and remodelling in animal models. The effect seems to increase over time and is impacted by the concentration used. However, included studies present a wide range of study methods. Future work should focus on consistent models and guidelines when developing a future clinical application of this element.

Cite this article: *Bone Joint Res* 2017;6:366–375.

Keywords: Biomaterials, Strontium, Bone

Article focus

- Systematic review
- Assess the *in vivo* effect of Sr-enriched biomaterials in bone formation and/or remodelling

Key messages

- Increased bone formation and/or remodelling in biomaterials enriched with Sr
- Sr effect is impacted by time and concentration

Strengths and Limitations

- First systematic review of Sr-enriched biomaterials
- Inclusion of studies with heterogeneous methods may impact comparability

Introduction

The treatment of fractures carries important challenges, especially when impaired bone healing or bone loss is present. Osteoporosis is increasingly found in Western countries as the population ages, leading to a significant rise in the incidence of specific fractures, where these problems are particularly evident.[1] New treatment options are needed to overcome the challenges associated with management of this condition.

Biological and synthetic bone grafts have been used to manage bone defects, and autografts are the current benchmark.[2] However, due to their limited availability, the morbidity associated with harvest surgery

and the relatively poor performance of synthetic materials, especially under osteoporotic conditions, there is a need for the development of effective and safer alternatives.[3,4] One proposed strategy has been the addition of osteo-inductive factors or osteoprogenitor cells to a bone substitute, in order to improve osteogenesis, particularly when impaired healing response is expected.[5-7]

Strontium (Sr) is a trace element that simultaneously stimulates bone formation and inhibits bone resorption.[8-10] Nevertheless, the cardiovascular safety of oral Sr ranelate is still a matter of concern as a small but significant increase in non-fatal myocardial infarctions has been reported,[11-13] leading to strict indications and restrictions for its use.[11]

Several pre-clinical studies, performed in both normal and osteoporotic animal models, were consistent with previous *in vitro* studies, showing the beneficial effect of Sr ranelate in increasing bone architecture and bone strength.[14-16] Accordingly, in order to enhance bone repair, Sr has recently been incorporated into different bone substitutes. This strategy aims to achieve a safer use of its osteoanabolic and anti-osteoclastic activity, as high concentrations are achieved locally, improving bone formation with less systemic impact. However, *in vivo* studies are scarce, and most reports do not present an adequate control group. Whether Sr-enriched materials are effective and safe is still a matter of debate, and more information on local Sr use is needed, with uniform criteria.

The aim of this study was to systematically review the *in vivo* effect of Sr in bone formation and/or remodelling, when incorporated into biomaterials.

Materials and Methods

A systematic search was performed in Pubmed and Scopus, using as a search strategy a combination of “Strontium”, “Bone Regeneration”, “Osteogenesis” (and similar terms such as ("Bone Substitutes" or Bone) and “Biomaterials” (and equivalents such as "Biocompatible Materials" or "Materials Testing" or "Tissue Scaffolds" or "Biomimetic Materials"). The search was limited by English language, human or other animal species (in Pubmed) and articles published after 1990 until July 2015. Additional papers were retrieved by non-systematic searches of relevant sources and screening of all retrieved article references (Fig. 1).

Increased bone regeneration was defined based on increased bone formation and/or increased bone remodelling. Two comparison groups were previously defined, as an experimental group (E), which received a Sr-enriched biomaterial for evaluation of bone support or regeneration, and a control group (C) that received a similar Sr-free material. The groups had to differ only in Sr addition to the biomaterial in order to be included. Subgroups were also defined when specific conditions were present in both experimental and control groups (such as osteoporosis).

The study was conducted in two phases (Fig. 1). In each phase, two independent reviewers (NN and DL) analysed the references and pooled according to predefined inclusion – A) studies with original data, B) on Sr doped materials, C) used for bone support or regeneration, D) performed *in vivo* – and exclusion criteria – E) articles without a control group only different from experimental on Sr addition to the biomaterial, F) on Sr usage only as a substitute on implant coating material, and G) if full-text not available (Fig. 1). In phase one, titles and abstracts were screened, and articles proceeded to the next phase upon inclusion of at least one reviewer. In phase two, full texts were assessed and disagreements were discussed between reviewers. When the full text was not available, authors were contacted and asked for a full-text copy. There was no article excluded due to unavailability of its full text.

Using an electronic form pre-developed by the authors, two investigators (NN and DL) performed data extraction. Qualitative results on the effect of Sr on bone regeneration were extracted independently of technique used for assessing Sr effect in each group. General results on implant effect were retrieved from individual papers, with data presented according to the amount of Sr in each biomaterial, time between material implantation and the analysis, and presence of concomitant conditions. The reported effect of Sr on bone formation, bone remodelling and its adverse effects were converted in a graphic summary table. Increased bone formation was considered as higher reported bone formation, total bone volume or other similar reference. Enhanced bone remodelling was defined as advanced maturation stage, higher biomaterial degradation, and central *versus* peripheral bone formation or similar.

When available, data on significance from each study were also gathered, with a statistically significant value defined as $p < 0.05$. Data on gene or protein expression were collected upon availability.

This systematic review was conducted based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines.[17] The PRISMA statement checklist is available as Supplementary material.

Results

A total of 572 references were retrieved after a literature search in Pubmed (210 references), and in Scopus (362 references), downgraded to 272 records after the application of exclusion criteria, the removal of duplicates, and the addition of hand and reference searches. In the title and abstracts selection phase, 231 records were excluded, mainly *in vitro* studies and studies on Sr’s usage as a coating material, rather than as an implant

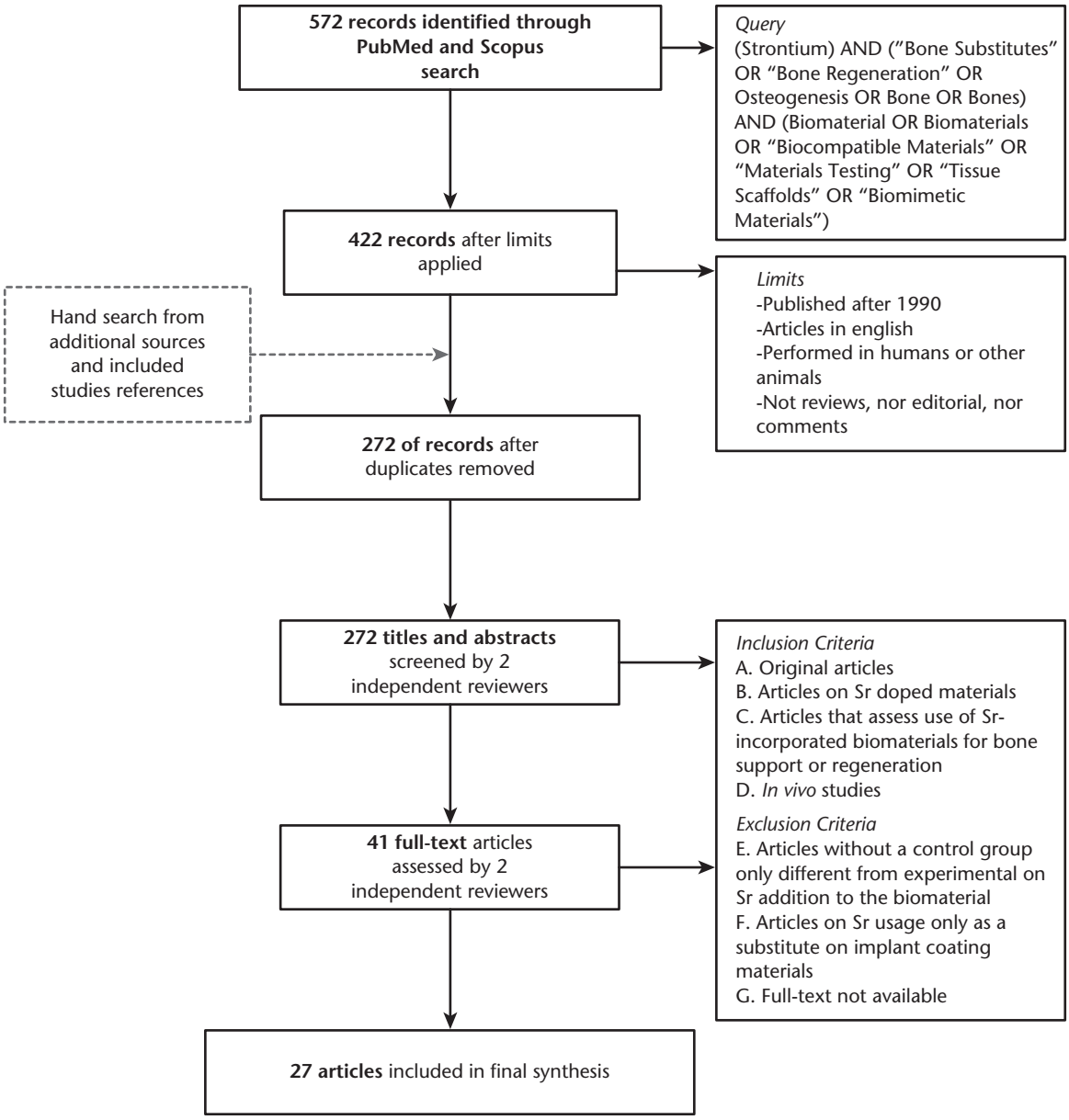

Fig.1

Flow diagram showing the study screening process.

for bone support or regeneration. In the full text selection phase, 41 papers were included. Four full texts were not available but were retrieved after contact with the authors. From these, 14 papers were excluded, mostly due to absence of control groups that received a Sr-free material, otherwise similar to the experimental material, and 27 articles were included in the final review (Fig. 1).[15,16,18-42]

General article information is available in Table I and general results on implant effect on Supplementary Table i. Rat models were used in 17 studies, nine were in rabbits and one in humans. The population of included studies ranged from four to 72 animals (Table I). Apart from two articles, the primary goal of the papers was to assess the effect of Sr-enriched materials in the models studied. The majority of bone defects were created in long bones, mainly in the femur (n = 18). Most of the defects were drilled and bilateral, with some studies using the same animal in the control and experimental groups. Three studies used segmental defects (Table I).

The major concomitant condition studied in the animal models was osteoporosis; two studies included models with and without osteoporosis, and nine included only osteoporotic animals. One study was performed on animals with osteonecrosis. In the remaining ten studies, all animals were healthy (Table I). The single article

Table I. Description of the sample and methods of the studies included

ID	Animal	n		Concomitant conditions	Defect			Material		Analysis
		E	C		Type	mm	Location	E	C	
Banarjee[18]	Sprague Dawley rats	8	4	H	Bilateral drilled	3	Distal Femur	ß-TCP-MgO/SrO cylinders	ß-TCP cylinders	Hist
Bose[19]	Sprague Dawley rats	4	4	H	Bilateral drilled	3	Distal Femur	ß-TCP-MgO/SrO cylinders	ß-TCP cylinders	Hist
Boyd[20]	Wistar rats	12	12	O/H	Unilateral drilled	1	Midshaft Femur	Sr-Bioactive glass	Bioactive glass	Hist
Cardemil[21]	Sprague Dawley rats	32	32	O/H	Bilateral drilled	2.3	Distal Femur	Sr-CP granules	HA granules	Hist*; G
Cheng[22]	Sprague Dawley rats	22	21	O	Unilateral wedge	4	Distal Femur	Sr-CPC Xerogel particles Sr-Fe foam	CPC Xerogel particles Fe foam	PET
Cheng[23]	Sprague Dawley rats	7	7	O	Unilateral wedge	4	Distal Femur	Sr-CPC	CPC	PET
Dagang[24]	New Zealand White rabbits	2	2	H	Unilateral drilled	2.2	Distal Femur	Sr-HA cement	HA cement	Hist
Gorustovich[25]	Wistar rats	15	15	H	Bilateral drilled	1.5	Tibia	Sr-Bioactive glass	Bioactive glass	Hist*
Gu[26]	New Zealand White rabbits	12	12	H	Unilateral segmental	15	Radius	Sr-CPP scaffold	Sr-CPP scaffold	Hist*
Lin[29]	Fisher rats	3	3	O	Drilled (2 defects)	5	Calvarius	Sr-Ca Silicate scaffold	Ca Silicate scaffold	Hist*; µ-CT
Mohan[30]	New Zealand White rabbits	6	6	H	Unilateral segmental	15	Midshaft Ulna	Sr-CP cylinders	HA cylinders	Hist*; µ-CT
Thormann[16]	Sprague Dawley rats	15	15	O	Unilateral wedge	4	Distal Femur	Sr-CPC	CPC	Hist*; G; Immuno
Tian[31]	New Zealand White rabbits	24	24	H	Unilateral segmental	15	Radius	Sr-CPP scaffold	CPP scaffold	Hist*; radiograph; Immuno
Wei[32]	Wistar rats	6	6	O	Bilateral drilled	3	Distal Femur	Sr Bioactive glass	Bioactive glass	Hist*; µ-CT
Xie[33]	New Zealand white rabbits	NI	NI	H	Unilateral	15	Femur	K/Sr-CPP scaffold	CPP scaffold	Hist*;
Zhao[35]	Sprague Dawley rats	6	6	H	Drilled (2 defects)	5	Calvarius	Sr Bioactive glass	Sr Bioactive glass	Hist*; µ-CT
Zhang[34]	Wistar rats	NI	NI	O	Bilateral drilled	3	Distal Femur	Sr Bioactive glass	Bioactive glass	Hist*; µ-CT; G; Immuno
Baier[15]	Sprague Dawley rats	30	30	O	Unilateral drilled	2	Distal Femur	Sr-CPC	CPC	Hist*
Izci[27]	Humans	4	4	Other†	Unilateral drilled	15	Cranium	Si-Sr-HA peg	Si-HA peg	µ-CT; Scintigraphy
Li[28]	Wistar rats	20	20	H	Bilateral drilled	3	Proximal Tibia	Sr-CaS paste	CaS paste	Hist*; µ-CT; X-ray
Jebahi[36]	Wistar rats	5	5	O	Unilateral drilled	3	Distal Femur	Sr Bioactive glass	Bioactive glass	Hist*
Jebahi[37]	Wistar rats	5	5	O	Unilateral drilled	3	Distal Femur	Sr Bioactive glass	Bioactive glass	Hist*
Kang[38]	Japanese White rabbits	18	18	ON	Unilateral drilled	3	Proximal Femur	Sr-CPP scaffold and MNCs	CPP scaffold and MNCs	Hist*; radiograph; Immuno
Tarafder[39]	Sprague Dawley rats	4	4	H	Bilateral drilled	3	Distal Femur	ß-TCP-MgO/SrO cylinders	ß-TCP cylinders	Hist*
Tarafder[40]	New Zealand White rabbits	2	2	H	Bilateral drilled	5.5	Distal Femur	ß-TCP-MgO/SrO cylinders	ß-TCP cylinders	Hist*; Immuno
Xie[41]	New Zealand White rabbits	9	9	H	Unilateral	15	Femur Shaft	K/Sr-CPP scaffold	CPP	Hist*; radiograph; Immuno
Zhang[42]	New Zealand White rabbits	6	6	H	Unilateral drilled	6	Distal Femur	Sr-Borate Bioactive glass	Borate Bioactive glass	Hist*

*Histomorphometry with Quantitative Analysis
†Neoplastic or Trauma conditions
H, Healthy; O, Osteoporotic; ON, Osteonecrosis; MNCs, Autologous Bone Marrow Mononuclear Cells; NI, No Information; TCP, Tricalcium Phosphate; CP, Calcium Phosphate; HA, Hydroxyapatite; CPC, Calcium Phosphate Cement; CPP, Calcium Polyphosphate; CaS, Calcium Sulfate; Hist, Histology/Histomorphometry; I, Imagiological; G, Gene Expression; PET, Positron-Emission Tomography Scan; µ-CT, micro-Computed Tomography; Immuno, Immunohistochemistry.

concerning humans included subjects who underwent a craniotomy due to different neoplastic and vascular conditions.

Time from implantation to analysis varied among the different studies, ranging from six days to 12 months. The studies used different materials. (Table I). Two sets of studies used similar materials; eight on bioactive glass and five on hydroxyapatite (HA)/calcium phosphate (CP) cements. Sr concentration in the E group ranged from 0.1% to 22% (Supplementary Table i). All but three studies performed histologic and/or histomorphometric analysis. Radiological analysis such as micro-CT, PET scan, radiograph or scintigraphy were used in 12 papers for imaging (Table I). Gene analysis and immunohistochemistry were available in two[21,31] and six[16,31,34,38,40,41] papers, respectively. Data on protein or gene expression are displayed in Figure 2.

Results stated by each paper on the effect of the Sr-enriched biomaterial in bone formation and/or remodelling are displayed in Supplementary Table i. Although five studies reported analysis with multiple Sr concentrations, only in three did the authors gather information on the comparison between materials with different Sr content. In two studies, a significant superior overall effect

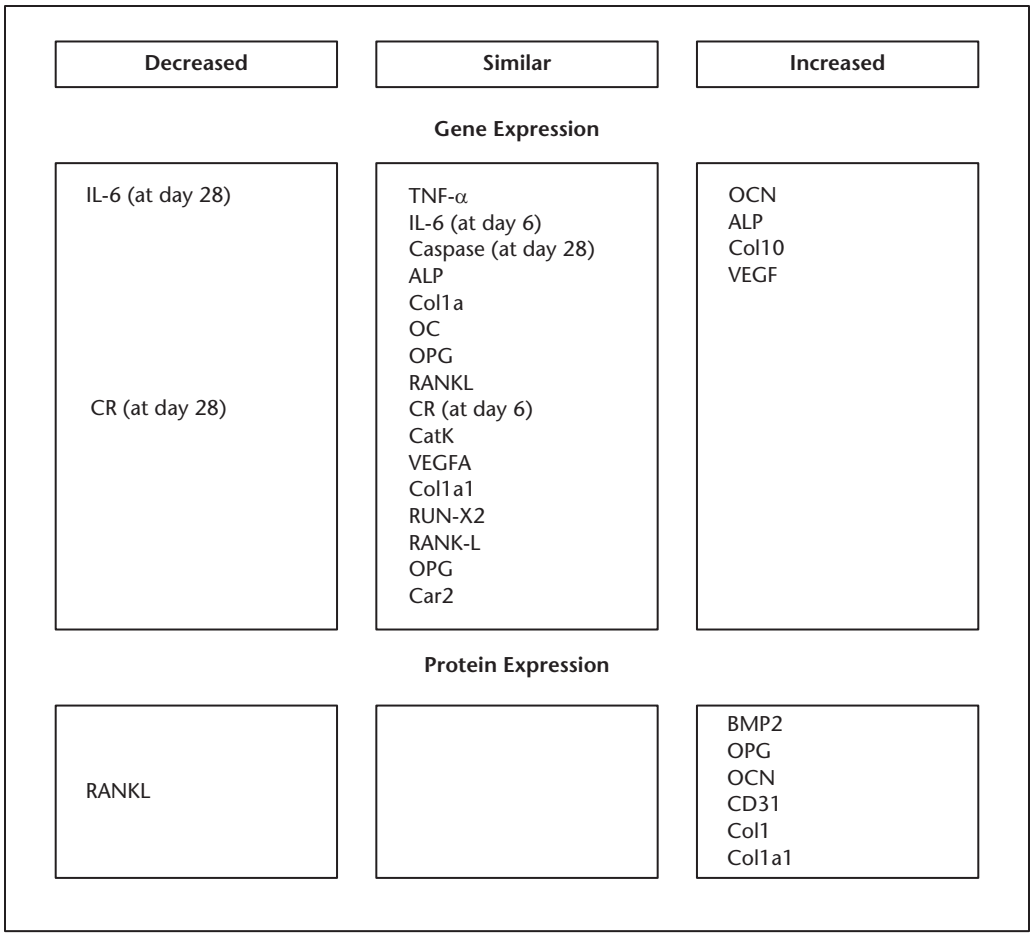

Fig. 2

Summary of study results on gene and protein expression. When different results from different study times were available, they were stated.

was found with materials enriched with higher Sr concentration; the other study that looked at various concentrations found non-significant differences. Only one article reported the differential effect between healthy specimens and those with osteoporosis, finding an increased effect in osteoporotic animals (Supplementary Table i).

A graphic analysis on the result of the effect of the studied biomaterial in bone formation and remodelling, along with a summary of adverse effects reported by each paper, is presented in Table II. Two articles studying bone formation reported a similar effect in the E and C groups; another one reported similar effects only in osteoporotic animals but an increased effect in healthy ones.[20] Baier et al[15] only found significant differences in the third month in both bone formation and remodelling. Cheng et al[22] studied three materials, with different compositions, but only one material, calcium phosphate cement (CPC), resulted in an increased effect on the experimental group. Apart from these, all other studies with analysis on bone formation found an increased effect of enriched material (Tables II and III).

Of the studies with reports on bone remodelling, four showed similar results in experimental and control, four found an increased effect in experimental only in late study phases, and 17 reported an increased effect in the experimental group in all studied times (Table III).

No study found a decreased effect of Sr addition in bone formation and/or regeneration when compared with controls. Overall, two articles had no report on bone remodelling, and another two did not report on bone formation.

Of the 17 articles with results on adverse effects of the implanted biomaterial, 13 reported similar local secondary effects in experimental and control. From these, 12 found no inflammatory reaction and one showed increased inflammation in both experimental and control. One article reported increased systemic effects of Sr application, with significantly raised levels of this ion in urine and blood samples, and the other three articles found no differences in systemic effects of Sr application.

Table II. Summary of general results on bone formation and bone remodelling from individual studies. Results are presented according to the content of Sr used in the biomaterial and the average time from implantation to evaluation

Article	Strontium content	Time	Bone formation	Bone remodelling	Adverse reaction	Inflammatory reaction
Bose[19]	1 wt%	4 wks	Increased	NI		
		8 wks	Increased	NI		
		12 wks	NI	Increased		
		16 wks	NI	Increased		
Tian[31]	1 wt%	4 wks	Increased*	Similar	L ↔	No
		12 wks	Increased*	Similar		
		16 wks	Increased*	Similar		
Xie[33]	2 wt%	4 wks	Increased*	Similar		
		8 wks	Increased	Similar		
		12 wks	Increased	Increased		
		Overall	Increased	Increased	L ↔	No
Dagang[24]	5/10 wt%	4/8/12/24 wks	similar	Increased	L ↔	No
Gorustovich[25]	6 wt%	30 days	NI	Similar	L ↔	No
Gu[26]	11.5 Ca/Sr MR	4 wks	NI	Increased	L ↔	No
		8 wks	NI	Increased	L ↔	No
		16 wks	Increased	Increased	L ↔	No
		Overall	Increased	Increased		
Banarjee[18]	0.25/1 wt%	4/16 wks	NI	Increased		
Li[28]	5/10 wt%	4 wks	Increased	Similar		
		8 wks	Increased	NI		
		12 wks	Increased	Increased		
Mohan[30]	1.67 (Ca+Sr)/P MR	4/12 wks	Increased*	Increased*	L ↔	No
Zhao[35]	10 wt%	8 wks	Increased*	Increased*		
Izci[27]	NI	3/6/12 mths	Increased	Similar	L ↔	No
Kang[38]	11.5 Ca/Sr MR	4/8/12 wks	Increased*	Increased*	L ↔	No
Tarafder[39]	1 wt%	4 wks	Increased*	Increased*	S (Similar Mg and Sr excretion in urine)	
		8 wks	Increased*	Increased*		
		12 wks	Increased*	Increased		
		16 wks	Similar	NI		
Tarafder[40]	1 wt%	8/12 wks	Increased*	Increased*		
Xie[41]	11.5 Ca/Sr MR	4 wks	Similar	Increased	L ↔	No
		8 wks	Increased	Increased	L ↔	No
		12 wks	Increased	Increased	L ↔	No
Zhang[42]	9 mol% SrO	4/8 wks	Increased*	Increased*	L ↔	No
Boyd[20]	0.14 SrO Mol Fract	4 wks	NI	Similar	L ↔	No
	0.28 SrO Mol Fract	4 wks	Increased	NI	L ↔	No
			Similar	NI		
Cardemil[21]	NI	6 days	Similar	NI	L ↔	Yes
		28 days	Similar	Increased*		
		6 days	Similar	NI		
		28 days	Similar	Increased*		
Wei[32]	5 wt%	2 wks	Similar	Similar		
		4 wks	Increased*	NI		
		8 wks	Increased*	Increased		
Thormann[16]	0.123 Sr/Ca MR	6 wks	Increased*	Increased*		
Zhang[34]	2.5 wt%	2 wks	Increased*	Increased*	S Increased* (Significant Sr increase in blood and urine samples)	
		4 wks	Increased*	Increased*		
		8 wks	Increased*	Increased / similar		
	5 wt%	2 wks	Increased*	Increased*		
		4 wks	Increased*	Increased*		
Baier[15]	NI	1 mth	Similar	Similar	S increased (Similar spine and contralateral BMD)	
		3 mths	Similar	Similar		
		6 mths	Increased*	Increased*		
Lin[29]	10 wt%	4 wks	Increased*	Increased*		
Cheng[22]	CPC – 8.36 wt%	6 wks	Increased	NI		
	Xerogel – 20 wt%		Similar	NI		
	Iron Foam – 22 wt%		Similar	NI		
Cheng[23]	8.36 wt%	6 wks	Increased	NI		
Jebahi[36]	0.1%	60 days	Increased	Increased	S (Similar blood cell counts and similar Ca and P blood levels)	
Jebahi[37]	0.1%	90 days	Increased*	Increased*	L ↔	No

Shaded cells represent results from osteoporotic models.
*Statistically significant difference between experimental and control
wt%, weight percentage; MR, Molar Ratio; Mol Fract, Molar Fraction; d, days; w, weeks; m, months; NI, No Information; L, Local; S, Systemic; CPC, Calcium Polyphosphate Cement; BMD, Bone Mineral Density
↔ Similar in experimental and control

Table III. Number of studies stating a specific result on bone formation and bone remodelling according to the time from implantation to evaluation. The single article on osteonecrosis was excluded from this analysis

Time (wks)	Healthy					Osteoporosis				
	No of articles	Bone formation		Bone remodelling		No of articles	Bone formation		Bone remodelling	
		Increased*	Similar†	Increased*	Similar†		Increased*	Similar†	Increased*	Similar†
1	1	1	0	0	0	1	1	0	0	0
2	0	0	0	0	0	2	1	1	1	1
4	14	3	8	5	8	6	3	3	1	3
6	0	0	0	0	0	3	2‡	3‡	0	1
8	10	1	8	1	7	3	0	3	0	3
12	10	1	8	2	7	2	1	1	1	1
16	5	1	2	1	3	0	0	0	0	0
24	2	1	1	1	1	1	0	1	0	1
48	1	0	1	1	0	0	0	0	0	0

†experimental *versus* control
*experimental *versus* control
‡Cheng, 2014[35] have different results with different materials

Discussion

This is the first systematic review that summarizes the *in vivo* effect in bone formation and remodelling of Sr-enriched biomaterials. Overall, Sr improves bone formation and remodelling, leading to a higher response when compared with similar Sr-free materials. Sr effect is present even in osteoporotic environments and some studies report greater effects in these models (Supplementary Table i). Our results are in agreement with other reviews on other enriching elements[43,44] and with previous *in vitro* results on Sr.[9,45-50]

Bone formation and remodelling: timing, models and health status. From the 25 articles with results on bone formation, 23 report some kind of improvement in the experimental group. Although not all state a benefit in all study points, we observed that the Sr effect appears mostly in the later stages of each study. In fact, a tendency to an increase in the number of studies reporting a stronger effect of Sr in bone formation in later study points is observed when analysing studies according to the time of evaluation, as seen in Table III. Conclusions from studies with earlier assessment points therefore may be premature to differentiate the bone reaction between experimental and control. This may explain why Cardemil et al[21] found no differences, since the study ended just four weeks after implantation. However, some authors reported a significant improvement in experimental even at weeks two and four. One can argue that response to Sr may be influenced by the amount of time that the bone is exposed to this component.

Few studies report similar effects on bone formation in experimental and control, and for each study time the number of studies reporting increased effect in experimental *versus* control is at least similar to the number of studies stating equal effects. When considering bone remodelling, fewer results are available, and the number of studies reporting increased bone formation and remodelling in experimental is only superior after six weeks. These results on bone formation and remodelling are valid, independent of the model's health status. This confirms previous reports on Sr, as a stimulator of bone differentiation and osteogenesis.[9,45-50] However, the optimal conditions for its usage are yet to be determined, in order to maximize its beneficial effects.

Moreover, no study showed decreased bone formation or remodelling in experimental, in any time period, for either healthy or diseased models. The presence of a beneficial effect of Sr even in osteoporotic models, may enhance its therapeutic value, since osteogenesis impairment is a major challenge in this condition.[4]

Biomaterials. We decided to consider Strontium Calcium Phosphate ($SrCaPO_4$) and Sr-HA as similar materials, since $SrCaPO_4$ results from incorporation of Sr into HA.[30] However, it is known that incorporation of Sr into HA may impact its solubility,[21] which may partially explain why only articles using biomaterials with Sr-HA showed no improved effect in E. Although Mohan et al[30] found a significant improvement in E using HA as a base material, we cannot make any comparison with the two other articles using HA since different defect models were used.

The rate of Sr release from the biomaterial was also identified as a possible factor impacting its activity, since osteoblast-like cells use the strontium released from the biomaterial to synthesize their mineralised extracellular matrix.[51] Thormann et al[16] showed higher Sr concentrations in zones of increased bone formation, supporting this finding. More studies on the relationship between Sr concentrations and bone formation are needed to clarify this theory.

Sr content. Our study also showed that even small amounts of Sr might be enough to have an impact on both bone formation and remodelling since some authors found significant differences with only 0.1% of this component.[36,37] However, two of the three authors who specifically compared different Sr percentages found an increased overall response with higher

concentrations,[24,34] confirming previous *in vitro* reports.[52] The other author reported similar results between E and C.[28] All three authors agree that the optimum dose of Sr is yet to be discovered, since it can impact both the bone response and material properties.[24,28,34]

Gene expression. The available information on Sr impact on gene expression can be seen in Figure 2. Broadly speaking, little variation was found but a decrease in the pro-inflammatory cytokine Il-6, a stimulator of osteoclast recruitment and bone reabsorption, related to altered bone metabolism, may help to explain the Sr effect as a promoter of bone formation and remodelling.[53] The increase in genes and proteins involved in these processes, such as osteocalcin and bone morphogenetic protein supports our reported results of Sr's bone forming effects.[53]

Side effects. As previously stated, Sr systemic side effects were responsible for a downgrading of its interest for the scientific community, and for a move to local application of this component.[11-13] Our review found only four studies on the systemic repercussion of local Sr application. While one study reported increased levels of Sr in both urine and blood samples,[34] another showed similar urinary excretion of this element.[39] The other two studies found no difference between experimental and control groups.[15,36] One study reported increased values of serum Sr, but no comparison with C was performed, and the authors state that the concentration was 100 times lower than that needed to produce systemic effects.[15] Nevertheless, the pathological effects of these findings are not well known and still a matter of debate for future studies. A total of 13 reported on local effects, but no differences were found from C, and only one study found an increased inflammatory reaction, both in experimental and control. Also, the short follow-up of most included studies impacts the ability to trace reliable conclusions on the adverse long-term effect of Sr.

Methodologies and limitations. The methods of the included studies were highly variable. First, different animal models were used, and only nine studies presented the same animal species. This can impact the results since it is known that both bone architecture and the regeneration process are different among species, posing problems when it comes to comparisons between studies.[54,55] Also, all studies in animals used small species, with known differences in bone macroscopic, microscopic and remodelling properties when compared with humans.[55] Although larger animals, like dog and sheep, present a more reliable model, they may pose more ethical, housing, handling and availability issues.[54,55] These variations between species may, at least partially, explain the different responses to the biomaterial found in the included studies. Only one article studied the application of Sr-enriched biomaterials in humans.[27] Although achieving an increased effect of Sr, the constraints of study design impact our ability to draw reliable conclusions.

Even in the same species, defects differ in size, type and location. All studies that created a segmental defect found an increased effect of Sr on both bone formation and remodelling independent of the study time. This did not happen with other types of defect. One may suppose that segmental defects, with a greater impact on bone macrostructure, may influence either bone response, with the possibility of stimulation of a quicker reaction, or the ability to retrieve reliable results in the early stages. Guidelines are not available regarding the appropriate defect needed for each case, and a recent study pointed out that differences in defect creation impact the bone response.[54] More accurate and homogeneous lines of conduct are essential for the future design of comparable and reproducible study models.

As stated before, time to healing evaluation is variable among studies. Previous reports stated that fracture consolidation with a neocortex consisting of woven and lamellar bone is usually completed in an average of five to six weeks. However, different models, and even different bone defects, can alter this timeline.[56]

Different methods for evaluation of response were found. Qualitative measurements, such as histology or imaging techniques, are observer-dependent subjective analyses, introducing bias to the reported results.[57] This may explain why Dagang, Kewei and Yong[24] found no significant differences between E and C since this study performed only a histological analysis.

Our review has other limitations. Only 27 studies were retrieved. From these, 20 presented numeric results from histological analysis, but a quantitative synthesis of data was not possible since only a few performed comparable measurements of bone formation and/or remodelling. These studies, along with those with only qualitative data, were included in the review, allowing a broad qualitative assessment of published studies but no meta-analysis was performed. The definition of bone formation and bone remodelling was subjective and different in each study. Two reviewers performed the selection of relevant results but the absence of strict definitions increases the risk of bias. Many studies, especially those with only qualitative data assessment, did not present the significance of the comparisons, increasing the subjectivity of their interpretation.

Future studies must follow appropriate protocols, and guidelines on results assessment for each technique should be drafted. The application of Sr in larger animal models, with longer follow-up times, is needed, with an appropriate monitoring of long-term local and systemic side effects. More studies on the comparison between diseased and healthy models, and between different Sr concentrations, would be of great importance to better understand the potentiality of this element.

In conclusion, Sr is an apparently safe and effective doping material for stimulating bone formation and remodelling. Its effect may be more pronounced and variable over time according to the concentration applied. Additionally, its benefit in osteoporotic models raises the possibility of its therapeutic value. However, the plethora of methods, measurements and protocols found in individual studies impacts the ability to perform a reliable data synthesis and analysis on Sr effect. It is important to develop adequate models and follow consistent guidelines of research in future studies, in order to better define the therapeutic application of this element.

References

1. **Alves SM, Economou T, Oliveira C, et al.** Osteoporotic hip fractures: bisphosphonates sales and observed turning point in trend. A population-based retrospective study. *Bone* 2013;53:430-436.
2. **Matassi F, Nistri L, Chicon Paez D, Innocenti M.** New biomaterials for bone regeneration. *Clin Cases Miner Bone Metab* 2011;8:21-24.
3. **Lin K, Liu P, Wei L, et al.** Strontium substituted hydroxyapatite porous microspheres: surfactant-free hydrothermal synthesis, enhanced biological response and sustained drug release. *Chem Eng J* 2013;222:49-59.
4. **Goldhahn J, Scheele WH, Mitlak BH, et al.** Clinical evaluation of medicinal products for acceleration of fracture healing in patients with osteoporosis. *Bone* 2008;43:343-347.
5. **Liu HY, Wu AT, Tsai CY, et al.** The balance between adipogenesis and osteogenesis in bone regeneration by platelet-rich plasma for age-related osteoporosis. *Biomaterials* 2011;32:6773-6780.
6. **Cao L, Liu G, Gan Y, et al.** The use of autologous enriched bone marrow MSCs to enhance osteoporotic bone defect repair in long-term estrogen deficient goats. *Biomaterials* 2012;33:5076-5084.
7. **Cho SW, Sun HJ, Yang JY, et al.** Human adipose tissue-derived stromal cell therapy prevents bone loss in ovariectomized nude mouse. *Tissue Eng Part A* 2012;18:1067-1078.
8. **Marie PJ, Felsenberg D, Brandi ML.** How strontium ranelate, via opposite effects on bone resorption and formation, prevents osteoporosis. *Osteoporos Int* 2011;22:1659-1667.
9. **Bonnelye E, Chabadel A, Saltel F, Jurdic P.** Dual effect of strontium ranelate: stimulation of osteoblast differentiation and inhibition of osteoclast formation and resorption in vitro. *Bone* 2008;42:129-138.
10. **Baron R, Tsouderos Y.** In vitro effects of S12911-2 on osteoclast function and bone marrow macrophage differentiation. *Eur J Pharmacol* 2002;450:11-17.
11. **Reginster JY, Neuprez A, Dardenne N, et al.** Efficacy and safety of currently marketed anti-osteoporosis medications. *Best Pract Res Clin Endocrinol Metab* 2014;28:809-834.
12. **Cooper C, Fox KM, Borer JS.** Ischaemic cardiac events and use of strontium ranelate in postmenopausal osteoporosis: a nested case-control study in the CPRD. *Osteoporos Int* 2014;25:737-745.
13. **Abrahamsen B, Grove EL, Vestergaard P.** Nationwide registry-based analysis of cardiovascular risk factors and adverse outcomes in patients treated with strontium ranelate. *Osteoporos Int* 2014;25:757-762.
14. **Hulsart-Billström G, Xia W, Pankotai E, et al.** Osteogenic potential of Sr-doped calcium phosphate hollow spheres in vitro and in vivo. *J Biomed Mater Res A* 2013;101:2322-2331.
15. **Baier M, Staudt P, Klein R, et al.** Strontium enhances osseointegration of calcium phosphate cement: a histomorphometric pilot study in ovariectomized rats. *J Orthop Surg Res* 2013;8:16.
16. **Thormann U, Ray S, Sommer U, et al.** Bone formation induced by strontium modified calcium phosphate cement in critical-size metaphyseal fracture defects in ovariectomized rats. *Biomaterials* 2013;34:8589-8598.
17. **Liberati A, Altman DG, Tetzlaff J, et al.** The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* 2009;339:b2700.
18. **Banerjee SS, Tarafder S, Davies NM, Bandyopadhyay A, Bose S.** Understanding the influence of MgO and SrO binary doping on the mechanical and biological properties of beta-TCP ceramics. *Acta Biomater* 2010;6:4167-4174.
19. **Bose S, Tarafder S, Banerjee SS, Davies NM, Bandyopadhyay A.** Understanding in vivo response and mechanical property variation in MgO, SrO and SiO_2 doped β-TCP. *Bone* 2011;48:1282-1290.
20. **Boyd D, Carroll G, Towler MR, et al.** Preliminary investigation of novel bone graft substitutes based on strontium-calcium-zinc-silicate glasses. *J Mater Sci Mater Med* 2009;20:413-420.
21. **Cardemil C, Elgali I, Xia W, et al.** Strontium-doped calcium phosphate and hydroxyapatite granules promote different inflammatory and bone remodelling responses in normal and ovariectomised rats. *PLoS One* 2013;8:e84932.
22. **Cheng C, Alt V, Pan L, et al.** Application of F-18-sodium fluoride (NaF) dynamic PET-CT (dPET-CT) for defect healing: a comparison of biomaterials in an experimental osteoporotic rat model. *Med Sci Monit* 2014;20:1942-1949.
23. **Cheng C, Alt V, Pan L, et al.** Preliminary evaluation of different biomaterials for defect healing in an experimental osteoporotic rat model with dynamic PET-CT (dPET-CT) using F-18-sodium fluoride (NaF). *Injury* 2014;45:501-505.
24. **Dagang G, Kewei X, Yong H.** The influence of Sr doses on the in vitro biocompatibility and in vivo degradability of single-phase Sr-incorporated HAP cement. *J Biomed Mater Res A* 2008;86:947-958.
25. **Gorustovich AA, Steimetz T, Cabrini RL, Porto López JM.** Osteoconductivity of strontium-doped bioactive glass particles: a histomorphometric study in rats. *J Biomed Mater Res A* 2010;92:232-237.
26. **Gu Z, Zhang X, Li L, et al.** Acceleration of segmental bone regeneration in a rabbit model by strontium-doped calcium polyphosphate scaffold through stimulating VEGF and bFGF secretion from osteoblasts. *Mater Sci Eng C Mater Biol Appl* 2013;33:274-281.
27. **Izci Y, Seçer HI, Ilica AT, et al.** The efficacy of bioceramics for the closure of burr-holes in craniotomy: case studies on 14 patients. *J Appl Biomater Funct Mater* 2013;11:e187-e196.
28. **Li X, Xu CP, Hou YL, et al.** A novel resorbable strontium-containing α-calcium sulfate hemihydrate bone substitute: a preparation and preliminary study. *Biomed Mater* 2014;9:045010.
29. **Lin K, Xia L, Li H, et al.** Enhanced osteoporotic bone regeneration by strontium-substituted calcium silicate bioactive ceramics. *Biomaterials* 2013;34:10028-10042.
30. **Mohan BG, Shenoy SJ, Babu SS, Varma HK, John A.** Strontium calcium phosphate for the repair of leporine (Oryctolagus cuniculus) ulna segmental defect. *J Biomed Mater Res A* 2013;101:261-271.
31. **Tian M, Chen F, Song W, et al.** In vivo study of porous strontium-doped calcium polyphosphate scaffolds for bone substitute applications. *J Mater Sci Mater Med* 2009;20:1505-1512.
32. **Wei L, Ke J, Prasadam I, et al.** A comparative study of Sr-incorporated mesoporous bioactive glass scaffolds for regeneration of osteopenic bone defects. *Osteoporos Int* 2014;25:2089-2096.
33. **Xie H, Wang Q, Ye Q, Wan C, Li L.** Application of K/Sr co-doped calcium polyphosphate bioceramic as scaffolds for bone substitutes. *J Mater Sci Mater Med* 2012;23:1033-1044.
34. **Zhang Y, Wei L, Chang J, et al.** Strontium-incorporated mesoporous bioactive glass scaffolds stimulating in vitro proliferation and differentiation of bone marrow stromal cells and in vivo regeneration of osteoporotic bone defects. *J Mater Chem B Mater Biol Med* 2013;1:5711-5722.
35. **Zhao S, Zhang J, Zhu M, et al.** Three-dimensional printed strontium-containing mesoporous bioactive glass scaffolds for repairing rat critical-sized calvarial defects. *Acta Biomater* 2015;12:270-280.
36. **Jebahi S, Oudadesse H, el Feki H, et al.** Antioxidative/oxidative effects of strontium-doped bioactive glass as bone graft. In vivo assays in ovariectomised rats. *J Appl Biomed* 2012;10:195-209.
37. **Jebahi S, Oudadesse H, Elleuch J, et al.** The potential restorative effects of strontium-doped bioactive glass on bone microarchitecture after estrogen-deficieny induced osteoporosis: physicochemical and histomorphometric analyses. *J Korean Soc Appl Biol Chem* 2013;56:533-540.
38. **Kang P, Xie X, Tan Z, et al.** Repairing defect and preventing collapse of femoral head in a steroid-induced osteonecrotic of femoral head animal model using strontium-doped calcium polyphosphate combined BM-MNCs. *J Mater Sci Mater Med* 2015;26:80.
39. **Tarafder S, Davies NM, Bandyopadhyay A, Bose S.** 3D printed tricalcium phosphate bone tissue engineering scaffolds: effect of SrO and MgO doping on in vivo osteogenesis in a rat distal femoral defect model. *Biomater Sci* 2013;1:1250-1259.
40. **Tarafder S, Dernell WS, Bandyopadhyay A, Bose S.** SrO- and MgO-doped microwave sintered 3D printed tricalcium phosphate scaffolds: mechanical properties and in vivo osteogenesis in a rabbit model. *J Biomed Mater Res B Appl Biomater* 2015;103:679-690.

41. Xie H, Wang J, Li C, et al. Application of strontium doped calcium polyphosphate bioceramic as scaffolds for bone tissue engineering. *Ceram Int* 2013;39:8945-8954.

42. Zhang Y, Cui X, Zhao S, et al. Evaluation of injectable strontium-containing borate bioactive glass cement with enhanced osteogenic capacity in a critical-sized rabbit femoral condyle defect model. *ACS Appl Mater Interfaces* 2015;7: 2393-2403.

43. Bose S, Roy M, Bandyopadhyay A. Recent advances in bone tissue engineering scaffolds. *Trends Biotechnol* 2012;30:546-554.

44. Qiu Z-Y, Noh I-S, Zhang S-M. Silicate-doped hydroxyapatite and its promotive effect on bone mineralization. *Front Mater Sci* 2013;7:40-50.

45. Chattopadhyay N, Quinn SJ, Kifor O, Ye C, Brown EM. The calcium-sensing receptor (CaR) is involved in strontium ranelate-induced osteoblast proliferation. *Biochem Pharmacol* 2007;74:438-447.

46. Atkins GJ, Welldon KJ, Halbout P, Findlay DM. Strontium ranelate treatment of human primary osteoblasts promotes an osteocyte-like phenotype while eliciting an osteoprotegerin response. *Osteoporos Int* 2009;20:653-664.

47. Brennan TC, Rybchyn MS, Green W, et al. Osteoblasts play key roles in the mechanisms of action of strontium ranelate. *Br J Pharmacol* 2009;157:1291-1300.

48. Choudhary S, Halbout P, Alander C, Raisz L, Pilbeam C. Strontium ranelate promotes osteoblastic differentiation and mineralization of murine bone marrow stromal cells: involvement of prostaglandins. *J Bone Miner Res* 2007;22: 1002-1010.

49. Zhu LL, Zaidi S, Peng Y, et al. Induction of a program gene expression during osteoblast differentiation with strontium ranelate. *Biochem Biophys Res Commun* 2007;355:307-311.

50. Fromigué O, Haÿ E, Barbara A, Marie PJ. Essential role of nuclear factor of activated T cells (NFAT)-mediated Wnt signaling in osteoblast differentiation induced by strontium ranelate. *J Biol Chem* 2010;285:25251-25258.

51. Kokesch-Himmelreich J, Schumacher M, Rohnke M, Gelinsky M, Janek J. ToF-SIMS analysis of osteoblast-like cells and their mineralized extracellular matrix on strontium enriched bone cements. *Biointerphases* 2013;8:17.

52. Christoffersen J, Christoffersen MR, Kolthoff N, Bärenholdt O. Effects of strontium ions on growth and dissolution of hydroxyapatite and on bone mineral detection. *Bone* 1997;20:47-54.

53. Rahman MS, Akhtar N, Jamil HM, Banik RS, Asaduzzaman SM. TGF-β/BMP signaling and other molecular events: regulation of osteoblastogenesis and bone formation. *Bone Res* 2015;3:15005.

54. Li Y, Chen S-K, Li L, et al. Bone defect animal models for testing efficacy of bone substitute biomaterials. *J Orthop Translat* 2015;3:95-104.

55. Pearce AI, Richards RG, Milz S, Schneider E, Pearce SG. Animal models for implant biomaterial research in bone: a review. *Eur Cell Mater* 2007;13:1-10.

56. Chakkalakal DA, Strates BS, Mashoof AA, et al. Repair of segmental bone defects in the rat: an experimental model of human fracture healing. *Bone* 1999;25:321-332.

57. Tadrous PJ. On the concept of objectivity in digital image analysis in pathology. *Pathology* 2010;42:207-211.

Funding Statement

- None declared.

Author Contribution

- N. Neves: Contributed to study conception and design, acquisition of data, analysis and interpretation of data, drafting of manuscript and critical revision.
- D. Linhares: Contributed to study conception and design, acquisition of data, analysis and interpretation of data and drafting of manuscript.
- G. Costa: Contributed to interpretation of data and critical revision.
- C. C. Ribeiro: Contributed to analysis and interpretation of data, drafting of manuscript and critical revision.
- M. A. Barbosa: Contributed to study conception and design, drafting of manuscript and critical revision.

ICMJE Conflicts of Interest

- None declared