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## Strontium modified calcium phosphate cements – approaches towards targeted stimulation of bone turnover

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Making use of the potential of calcium phosphates to host a variety of ions in their crystal lattice, ion substitution of calcium phosphate bone cements has become the subject of intense investigations in the last few years, since this approach allows one to stabilize a bone defect and to locally deliver therapeutic ions into a specific defect site at the same time. In this respect significant attention has been given to strontium ions ( $\text{Sr}^{2+}$ ) lately. Strontium possesses the unique potential to both stimulate new bone formation and inhibit cell-driven bone resorption and thus has been used successfully in systemic osteoporosis therapy. Strontium doping of calcium phosphate bone cements might allow making use of this dual effect to promote local bone defect healing. The goal of this review is to provide an overview of different routes that have been employed to obtain strontium-containing calcium phosphate bone cements and describe their material characteristics as well as their biological properties based on cell culture and animal studies.

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### 1. Introduction

Biomaterials, in terms of materials intended to come into contact with living tissue or to be implanted into a living

organism, are usually described on the basis of their specific degree of biocompatibility. Although various definitions of “biocompatibility” exist, most of them are based on absence of negative tissue reaction and/or formation of a fibrous interface.<sup>1</sup> For example, materials in contact with bone tissue are required to allow the formation of a strong bone–material interface in order to support the mechanical integrity of the host bone. Biomaterials for hard tissue repair have been in the

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include the development of biomaterials, with focus on hard tissue reconstruction, calcium phosphates and tissue engineering as well as rapid prototyping of biomaterials.

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*(CRTD). His multidisciplinary group is focused on novel therapeutic options for musculoskeletal diseases including biomaterial development, stem cell research and tissue engineering. His lab also has started to explore new technologies of additive manufacturing and biofabrication for medical applications.*

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focus of research for many years now. Calcium phosphates (CaP), in particular hydroxyapatite (HA) and brushite (DCPA), are widely used in bone graft applications as well as in coatings of metallic implants, since their chemical resemblance to the mineral part of the natural bone offers excellent biocompatibility.<sup>1,2</sup> Since some mixtures of calcium phosphates can, once in contact with water, undergo a hydraulic setting reaction into either HA or DCPA, a number of self-setting cements have been developed of which many are in clinical use today. Calcium phosphate bone cements (CPC) in general can be shaped into a specific bone defect during implantation, are highly biocompatible and are bio-resorbable.<sup>3</sup> However, only recently concepts beyond “simple” biocompatibility have been developed: on the one hand tissue engineering aims at the implantation of cell seeded, functional biomaterial scaffolds; on the other hand materials with an intrinsic potential to influence the host tissue have been proposed. Such “third generation biomaterials” are meant to (locally) evoke a specific cellular response based on the cell-material interaction.<sup>4</sup> These may be materials that contain and locally release drugs upon implantation as well as materials which are composed of biologically effective components that are released as soon as the implants degrade in the body. Much effort has been made to investigate the potential of CaP and CPC comprising biologically potent ions, such as silicate,<sup>5–8</sup> magnesium<sup>9–11</sup> and many others.<sup>2,12</sup> Whilst drug delivery from CaP bone cements has been reviewed in detail recently,<sup>13</sup> this study is focussed on cements comprising biologically highly effective strontium ions (Sr<sup>2+</sup>). Strontium has been proposed to possess both a bone formation stimulating and at the same time anti-resorptive effect and is thus used in systemic osteoporosis therapy.<sup>14</sup>

Our aim is to provide a rationale for the development of Sr-laden CaP bone cements for the treatment of bone defects, an overview of different routes that have been employed to obtain such cements as well as their material characteristics and *in vitro* and *in vivo* properties.

### 1.1 Strontium in bone

Strontium (Sr) is an alkaline earth metal accounting for approx. 0.02–0.03% of the earth's crust.<sup>15,16</sup> Its concentration in drinking water varies with geographic region, but is generally low, for example less than 1 mg ml<sup>-1</sup> in the United States.<sup>16</sup> A typical diet contains about 2–4 mg Sr per day,<sup>15,16</sup> and the secretion of Sr exceeds that of Ca, accounting for a fairly low bioavailability of orally ingested Sr of ~20%.<sup>17</sup> Since Sr<sup>2+</sup> is generally regarded as bone-seeking and thus could remain in the body for a long time once ingested, the interest in strontium metabolism arose from radioactive <sup>90</sup>Sr atmospheric contamination following the nuclear weapon tests since 1945.<sup>15</sup> Radioactive isotopes of Sr have also been used for diagnostic purposes.<sup>15</sup> Strontium was discovered to follow the metabolic pathways and signalling principles known for calcium, although the response to Sr<sup>2+</sup> tends to be weaker. Strontium shows a comparable protein binding capacity to that of Ca and is mostly deposited in the bone mineral.<sup>15</sup> In 1952 Shorr and Carter described an increased calcium absorption and thus bone formation under the influence of strontium lactate (SrC<sub>6</sub>H<sub>12</sub>O<sub>6</sub>·xH<sub>2</sub>O).<sup>18</sup> A first clinical trial

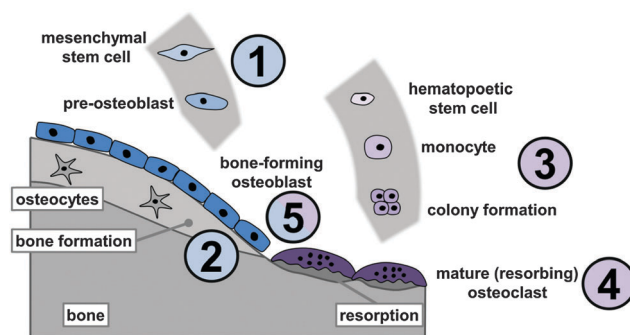


Fig. 1 Effects of Sr<sup>2+</sup> ions on bone metabolism: stimulation of osteoblast-precursor proliferation and osteogenic differentiation (1), increase of bone mineralisation by osteoblasts (2), reduced osteoclast-precursor recruitment and osteoclastogenesis (3), decreased resorption activity and increased apoptosis of mature osteoclasts (4) as well as interaction with the osteoblast/osteoclast paracrine signalling (5).

where patients with postmenopausal osteoporosis received up to 1.75 g d<sup>-1</sup> Sr in the form of strontium lactate over a period between 3 months and 3 years showed an increase in bone mass under Sr administration and a potential benefit in osteoporosis therapy without any side effects.<sup>19,20</sup> Systematic studies revealed the bone conserving effect of divalent strontium in ovariectomized (OVX) rats,<sup>21</sup> until two clinical phase III trials showed a significant increase in bone mass under the influence of 2 g d<sup>-1</sup> S-12911 (strontium ranelate, SrRan) in postmenopausal women.<sup>22,23</sup>

Unlike most other drugs used in osteoporosis therapy, Sr exerts a dual effect on bone remodelling, being able to enhance osteoblast activity and thus increase new bone formation and at the same time inhibit osteoclast activity and thus reduce cellular bone resorption (Fig. 1).<sup>24–27</sup> Several *in vitro* studies confirmed this twofold effect of Sr on bone metabolism. For example, osteoblast precursor proliferation and expression of extracellular matrix proteins are increased by ~10<sup>-3</sup> mM Sr<sup>2+</sup>, and osteoblast-mediated bone formation is stimulated in the presence of 2–5 μg ml<sup>-1</sup> (2.3–5.7 × 10<sup>-3</sup> mM) Sr<sup>2+</sup>.<sup>28,29</sup> Although the exact mechanism of how Sr affects bone cells remains unknown, it has been proposed that Sr acts on the *calcium sensing receptor* (CaSR) expressed by bone cells as a calcium-like entity and thus interacts with signalling pathways associated with the Ca-driven regulation of bone metabolism.<sup>30,31</sup> Furthermore, an increase of β-catenin expression indicating an enhanced transcription of osteogenic factors, a decrease in Wnt-pathway inhibitors as well as an enhanced prostaglandin-expression under the influence of Sr have been described.<sup>30,32</sup> Interestingly, similar concentrations of Sr inhibit the formation of osteoclasts and their resorption activity through either paracrine signalling<sup>33–35</sup> or even the induction of osteoclast apoptosis.<sup>36</sup>

### 1.2 Calcium phosphate bone cements

First described by LeGeros as well as Brown and Chow in the 1980s, calcium phosphate cements (CPC) have become a frequently used biomaterial in clinical application today.<sup>37–39</sup> CPC are hydraulic cements and are usually prepared by mixing



Table 1 Calcium phosphate species frequently used to prepare CPC<sup>3,40</sup>

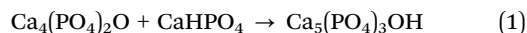
Name	Abbreviation	Chemical formula	Ca/P ratio	pH <sup>a</sup>
Calcium dihydrogen phosphate monohydrate	MCPM	Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> ·H <sub>2</sub> O	0.5	0–2.0
Calcium dihydrogen phosphate (anhydrous)	MCPA	Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub>	0.5	—
Calcium hydrogen phosphate dihydrate (brushite)	DCPD	CaHPO <sub>4</sub> ·2H <sub>2</sub> O	1.0	2.0–6.0
Calcium hydrogen phosphate anhydrous (monetite)	DCPA	CaHPO <sub>4</sub>	1.0	—
Octacalcium phosphate	OCP	Ca <sub>8</sub> (HPO <sub>4</sub> ) <sub>2</sub> (PO <sub>4</sub> ) <sub>4</sub> ·5H <sub>2</sub> O	1.33	5.5–7.0
α-Tricalcium phosphate	α-TCP	α-Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	1.5	—
β-Tricalcium phosphate	β-TCP	β-Ca <sub>3</sub> (PO <sub>4</sub> ) <sub>2</sub>	1.5	—
Amorphous calcium phosphate	ACP	Ca <sub>x</sub> H <sub>y</sub> (PO <sub>4</sub> ) <sub>z</sub> ·nH <sub>2</sub> O n = 3–4.5	—	> 5
Ca-deficient hydroxyapatite	CDHA	Ca <sub>10–x</sub> (HPO <sub>4</sub> ) <sub>x</sub> (PO <sub>4</sub> ) <sub>6–x</sub> (OH) <sub>2–x</sub> (0 < x < 1)	—	6.5–9.5
Hydroxyapatite	HA	Ca <sub>5</sub> (PO <sub>4</sub> ) <sub>3</sub> OH	1.67	> 9.5
Tetracalcium phosphate	TTCP	Ca <sub>4</sub> (PO <sub>4</sub> ) <sub>2</sub> O	2.0	—

<sup>a</sup> Stability range in aqueous media at 25 °C.

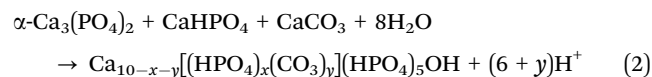
one or more calcium orthophosphate powders (the so-called precursor) with an aqueous liquid. A list of CaP phases frequently used to prepare CPC is given in Table 1.

Upon mixing of the precursor with the liquid phase a paste forms that will self-set and harden *via* a hydraulic reaction, characterised by dissolution of the precursor components and the precipitation of a thermodynamically more stable setting product. Mechanical integrity results from the entanglement of the newly formed crystals.<sup>41</sup> Although a large number of CPC formulations have been proposed, all of them set into one of two possible end products at 37 °C: hydroxyapatite (Ca<sub>5</sub>(PO<sub>4</sub>)<sub>3</sub>(OH), HA), as the most stable phase at pH values >4.2, or brushite (CaHPO<sub>4</sub>·2H<sub>2</sub>O, DCPD), which is the result of the setting reaction at pH <4.2.<sup>3</sup> The chemical nature of the reaction can, in principle, be either an acid–base reaction (*e.g.* the brushite-forming reaction of the acidic MCPM with slightly basic β-TCP) or the conversion of a metastable phase, such as amorphous calcium phosphate (ACP) or α-TCP, into a more stable one.<sup>3,40</sup> The setting reaction will lead to the hardening of the paste within a characteristic time span (setting time).

**Apatite-forming cements.** Since the mineral part of natural bone is composed of Ca-deficient hydroxyapatite containing a variety of substitutions such as carbonate, magnesium, sodium, *etc.* (referred to as “bone apatite”),<sup>1</sup> the choice of HA-forming cements for bone repair is self-evident. The classical apatite-forming cement of Brown and Chow is based on the reaction of basic tetracalcium phosphate with calcium hydrogen phosphate (eqn (1)).<sup>38</sup>



Numerous other cement formulations have been proposed based on the setting reaction of one more acidic and one more basic CaP component, *e.g.* combinations of α-TCP with MCPM and CaO or the reaction of TTCP, β-TCP and MCPM.<sup>42,43</sup> Driessens and co-workers developed a cement setting into carbonated, Ca-deficient HA more similar to the natural bone apatite (eqn (2)).<sup>44</sup>



One second category is cements derived from a one-component precursor that set *via* the conversion of a less stable CaP phase

into hydroxyapatite upon mixing with water, *e.g.* the hydrolysis of α-TCP and its re-precipitation into CDHA or the conversion of ACP into HA.<sup>45,46</sup> Apatite-forming cements possess a very low solubility according to their setting product: CDHA has a slightly higher solubility than stoichiometric HA (Table 2). This leads to relatively low physico-chemical degradation.<sup>47</sup> However, apatite cements can be resorbed *in vivo* *via* cellular activity (osteoclastic resorption),<sup>48</sup> although the degree of resorption seems to depend on the actual setting product (HA or CDHA), the respective degree of crystallinity and the porosity. Mechanical characterisation revealed that compressive strength values of completely set HA-forming cements are up to 70–80 MPa.<sup>40,41</sup>

**Brushite-forming cements.** Invented by Mirtchi and Lemaître in 1987, brushite is the second possible end product of hydraulic CPC reactions.<sup>57</sup> The most common formulation is based on an equimolar mixture of β-TCP and MCPM as given in eqn (3).<sup>58</sup>

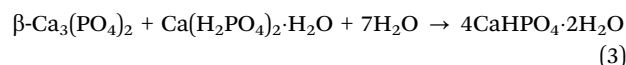


Table 2 Solubility of various CPC and SrCPC components and setting products

Components with low solubility			
Formula	Solubility at 25 °C [–log(K <sub>sp</sub> )]	Solubility at 25 °C [mg l <sup>–1</sup> ]	Ref.
α-TCP	25.5	2.5	49 and 50
β-TCP	28.9	0.5	49 and 50
CaHPO <sub>4</sub> (DCPA)	6.90	48	49 and 50
SrHPO <sub>4</sub> (DSPA)	6.7–6.9	63	51
CaCO <sub>3</sub>	4.90	14	52 and 53
SrCO <sub>3</sub>	8.10	10	52 and 53
CDHA	~85.1	~9.4	3 and 49
HA	58.4–116.8	0.3	3, 49 and 50
DCPD/brushite	6.59	88	49 and 50
SrHA (0.3–10% Sr <sup>2+</sup> )	115–117	—	54 and 55
Sr-brushite	Increased (comp. to brushite)	—	56
TTCP	38–44	0.7	49 and 50
Highly soluble components			
Formula	Solubility at 25 °C [g l <sup>–1</sup> ]		Ref.
SrCl <sub>2</sub>	1060		52 and 53
Sr(NO <sub>3</sub> ) <sub>2</sub>	800		52



Other formulations comprise  $\beta$ -TCP and phosphoric acid or MCPM, tetracalcium phosphate and CaO, but are all based on an acid–base reaction mechanism.<sup>59,60</sup> In general, brushite cements possess a much higher solubility (Table 2) and thus a higher degradation rate than HA under physiological conditions.<sup>61</sup> Moreover, because of its metastable nature, at  $\text{pH} > 6$  brushite hydrolyses into CDHA over time, which may lead to the release of orthophosphoric acid that could cause the inflammatory tissue response sometimes reported after implantation of large quantities of brushite-forming cements.<sup>62</sup> Brushite can also be resorbed by cellular activity *in vitro* and *in vivo*.<sup>61,63</sup> However, setting of brushite cements takes place at low pH values and such acidification may not only complicate the cellular reaction *in vitro* but also hamper tissue integration *in vivo*. Brushite cements are mechanically slightly weaker than apatite forming cements. Compressive strength values of up to 60 MPa have been reported.<sup>40</sup>

## 2. Strontium-modified CaP cements

The potential of CPC to host various ions like  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{CO}_3^{2-}$ ,  $\text{Cl}^-$ , *etc.* has been described before.<sup>41</sup> Given the ability of some ions (calcium, phosphate, strontium, silicate, zinc and magnesium) to trigger bone cell response and their advantages over bioactive proteins like growth factors, such as lower cost and better stability, the local release of therapeutically potent ions from an implanted bone cement could have big impact on bone healing strategies.<sup>13</sup> In 2000, Li *et al.* published the development of a SrHA (strontium-substituted HA) containing resin-based bone cements with good mechanical and *in vitro* characteristics.<sup>64</sup> Recently, the synthesis and characterisation of Sr-containing CPC (SrCPC) has become the focus of many studies. From a crystallographic point of view, the basis of all those developments is that strontium, as some other divalent cations, can be integrated into the crystal lattice of calcium phosphates on  $\text{Ca}^{2+}$  positions over a wide range of concentrations preserving the respective crystal structure.<sup>56,65,66</sup> It was reported that  $\text{Sr}^{2+}$  can substitute  $\text{Ca}^{2+}$  in a number of CaP phases, including amorphous calcium phosphate, hydroxyapatite, octacalcium phosphate and brushite.<sup>67</sup> In hydroxyapatite,  $\text{Sr}^{2+}$  can occupy either the Ca(I) or Ca(II) position, although Ca(I) is only preferred at very low levels of substitution.<sup>68</sup> Due to its larger ionic radius ( $\text{Ca}^{2+}$ : 148 pm;  $\text{Sr}^{2+}$ : 158 pm in hexagonal hydroxyapatite)<sup>69</sup> strontium substitution causes a linear increase of apatite lattice parameters.<sup>54,68,70</sup> At higher concentrations Sr substitution has been shown to decrease the crystallinity.<sup>68,71</sup> In the case of brushite, substitution of  $\text{Ca}^{2+}$  by  $\text{Sr}^{2+}$  ions is not limited to specific Ca sites and causes an expansion of the unit cell volume and thus a shift in the X-ray diffraction peaks towards lower diffraction angles.<sup>67</sup> The same effect can be found in  $\alpha$ - and  $\beta$ -TCP.<sup>72–74</sup>  $\beta$ -TCP was shown to possess the ability to host up to 80 at% strontium which is accompanied by a linear increase of the lattice parameters.<sup>72</sup> Lattice distortion induced by the larger  $\text{Sr}^{2+}$  ions has been found to be in charge of the increased solubility of substituted apatite and brushite.<sup>55,56</sup> However, even fully substituted apatite

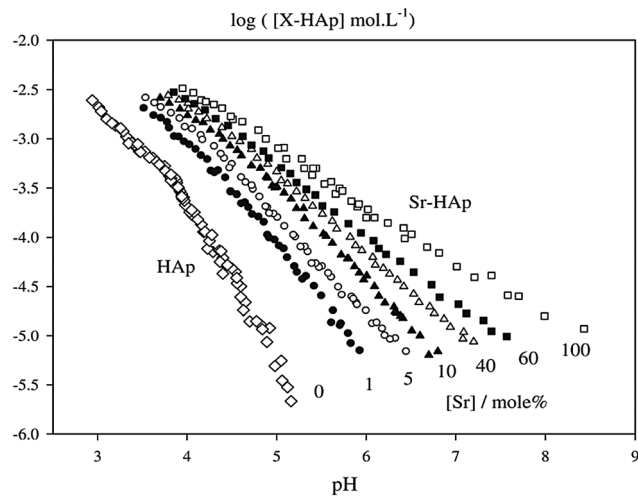


Fig. 2 Solubility isotherms of hydroxyapatite ("HAp") and "Sr-HAp" with different degrees of Sr-substitution. Reprinted from H. B. Pan, *et al.*, Solubility of strontium-substituted apatite by solid titration, *Acta Biomater.*, 2009, 5(5), 1678–1685, with permission from Elsevier.

(Ca free strontium-apatite) possesses a solubility higher than that of pure HA (Fig. 2) despite being as highly ordered as HA.<sup>55</sup> Thus substitution could help to enhance *in vivo* degradability and, consequently, osseointegration of SrCPC. Moreover, Sr can affect the conversion of  $\alpha$ -TCP to CDHA and could therefore be used as a tool to modulate the setting characteristics.<sup>75</sup> In particular,  $\text{Sr}^{2+}$  (and other divalent cations such as  $\text{Mg}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Cu}^{2+}$ )<sup>76–78</sup> has been shown to block  $\alpha$ -TCP hydrolysis. In the context of CPC this could decelerate conversion into CDHA and thus setting, a principle that was recently employed to prevent aqueous  $\alpha$ -TCP pastes from setting and thus to obtain storable cement precursor pastes that can be activated upon mixing with a second,  $\text{Ca}^{2+}$  containing liquid.<sup>78</sup> The presence of  $\text{Sr}^{2+}$  has further been shown to affect the rate of apatite nucleation;<sup>79</sup> in particular, under the influence of  $\text{Sr}^{2+}$  apatite deposition on HA samples from a  $\text{Ca}^{2+}$  and  $\text{Sr}^{2+}$  containing solution has been demonstrated, but, interestingly, this effect was less pronounced if the substrate was SrHA.<sup>54</sup>

In principle there are four possible ways to obtain strontium containing or strontium substituted calcium phosphate cements: one can (a) add Sr-containing phases to an existing cement system, (b) substitute calcium phases in the cement precursor powder by their strontium analogues (*e.g.* substitution of  $\text{CaHPO}_4$  by  $\text{SrHPO}_4$ ), (c) use Sr-substituted reactive CaP phases as a component in the precursor (*e.g.* Sr-substituted  $\alpha$ -TCP) or (d) use a strontium-salt-containing solution as liquid phase during cement paste preparation (Fig. 3).

As for CPC, setting of SrCPC depends on the solubility of the precursor powder components.<sup>80</sup> During setting, the Sr component can either act as an inert filler or undergo dissolution and re-precipitation as the other precursor components or crystallise from the liquid. Besides the respective solubility of the precursor phases the overall (Ca + Sr)/P ratio of the powder has to be taken into account, since both an excess or shortage of cations ( $\text{Ca}^{2+}$ ,  $\text{Sr}^{2+}$ ) can inhibit the setting reaction, alter the



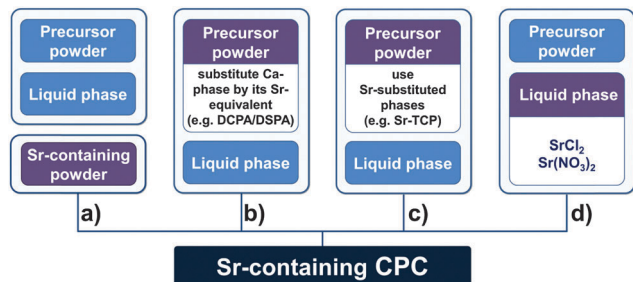


Fig. 3 Processing routes towards SrCPC: addition of Sr-containing reagents (a), substitution of reagents by their Sr-equivalents (b), use of Sr-substituted reagents in the precursor (c) and use of a Sr-containing liquid (d).

setting product or decrease the reaction yield. Regarding the end-product of the setting reaction, cement matrices with embedded Sr-rich clusters (those systems where the Sr component does not undergo complete dissolution) can be distinguished from monophasic apatite matrices with  $\text{Sr}^{2+}$  substituted into the crystal lattice. Another mechanism has been proposed by Kuang *et al.*, who attribute the initial hardening of the cement to a fast chelate reaction of Ca/Sr ions and carboxy groups from carboxylic acids (such as citric acid) in the cement liquid.<sup>81,82</sup>

Table 3 summarises different approaches towards preparation of SrCPC and indicates the mono- or biphasic nature of the set cement.

## 2.1 Apatite-forming formulations

Certainly the most straightforward way to introduce Sr into a cement is the addition of a Sr-containing phase to the cement powder. Wang *et al.* intermixed  $\text{SrCO}_3$  with an ACP/DCPD cement precursor by intense milling and found a retarded setting of the cement into HA. The set cement was characterised by a smaller grain size, which can be attributed to the milling process. However,  $\text{SrCO}_3$  was still detected in the XRD spectra of the set cements and thus Sr did not quantitatively

substitute into the apatite lattice.<sup>87</sup> Another cement was obtained by addition of  $\text{SrCO}_3$  particles with a size of  $\sim 10 \mu\text{m}$  to a cement containing  $\alpha$ -TCP, DCPA, HA and  $\text{CaCO}_3$ . Here, no additional milling step was used, and therefore the set cement was characterised by relatively large  $\text{SrCO}_3$  clusters embedded in an apatite matrix.<sup>86</sup> Furthermore, with the aim of mechanical reinforcement of the cement, SrHA whiskers prepared using a hydrothermal synthesis route were added to a  $\beta$ -TCP/DCPD cement precursor by Shen and co-workers.<sup>92</sup>

Another approach to obtain Sr-containing HA cements is the substitution of Ca-phases by their Sr analogues. For example, using the cementing reaction of DCPA/TTCP into hydroxyapatite upon mixing with phosphoric acid, DCPA can be substituted by its strontium analogue strontium hydrogen phosphate ( $\text{SrHPO}_4$ , DSPA). In this way, Sr-substituted apatite cements with a  $\text{Sr}/(\text{Sr} + \text{Ca})$  ratio of up to 0.1 were prepared by Guo *et al.*<sup>83,84</sup>

Since mixtures of DCPD and  $\text{CaCO}_3$  can set into HA, one strategy to obtain SrHA cements is to replace  $\text{CaCO}_3$  in the precursor by  $\text{SrCO}_3$ . Following this approach, precursors composed of DCPD and various  $\text{CaCO}_3$ - $\text{SrCO}_3$  mixtures were prepared by Tadier *et al.* to obtain cements with up to 8 wt% Sr.<sup>85</sup> In contrast to the above-mentioned systems, the setting product was biphasic: the set cement was composed of a mixture of hydroxyapatite and  $\text{SrCO}_3$  and no SrHA could be detected. Similarly, no homogeneous Sr-distribution was achieved by a precursor mixture of DCPD and amorphous calcium phosphate (ACP) in which the latter was partially replaced by amorphous strontium phosphate (ASP). Although total  $\text{Sr}/(\text{Sr} + \text{Ca})$  ratios between 0.025 and 0.1 could be obtained the set cement was composed of coexistent HA and SrHA.<sup>89</sup> On the other hand, in an  $\alpha$ -TCP/DCPA/HA/ $\text{CaCO}_3$  cement system setting into CDHA, substitution of  $\text{CaCO}_3$  by  $\text{SrCO}_3$  in the precursor powder mixture was shown to result in the formation of a monophasic, Sr-substituted apatite matrix and enhanced mechanical properties after setting.<sup>86</sup> Baier *et al.* replaced both DCPA and  $\text{CaCO}_3$  by DSPA and  $\text{SrCO}_3$  in an  $\alpha$ -TCP cement and obtained good *in vivo* results; however, no material

Table 3 Selected Sr-containing cement formulations and setting products (m: monophasic cement matrix, b: biphasic cement matrix)

Sr-source	Solid or liquid	Precursor phases	Setting products	Mono or biphasic	Ref.
DSPA	s	TTCP, DCPA, DSPA	SrHA	m	83 and 84
$\text{SrCO}_3$	s	$\text{CaCO}_3$ , $\text{SrCO}_3$ , DCPD	HA, $\text{SrCO}_3$	b	85
$\text{SrCO}_3$	s	$\alpha$ -TCP, HA, DCPA, $\text{CaCO}_3/\text{SrCO}_3$	HA, $\text{SrCO}_3$	b	86
$\text{SrCO}_3$	s	ACP, DCPD, $\text{SrCO}_3$	HA, $\text{SrCO}_3$	b	87
$\text{SrCO}_3$	s	DCPD, CaO/ $\text{SrCO}_3$	SrHA	b	88
ASP	s	ACP, ASP, DCPD	SrHA, HA	b	89
SrACP	s	SrACP, DCPA	SrHA, $\text{SrCO}_3$ after ageing SrHA	b	90
Sr- $\alpha$ -TCP	s	Sr- $\alpha$ -TCP	SrHA	m	73
$\text{SrCO}_3 + \text{SrHPO}_4$	s	$\alpha$ -TCP, DSPA, $\text{SrCO}_3$ , HA	Not characterized	—	91
DSPA	s	TTCP, DCPA/DSPA <sup>a</sup>	SrHA	m	81
SrHA W <sup>b</sup>	s	$\beta$ -TCP, DCPA, Sr-HA-whiskers	Not characterized	b	92
$\text{SrCl}_2$	l	$\alpha$ -TCP/gelatin, DCPD	SrCDHA	m	75
$\text{SrCl}_2$	l	$\text{CaCO}_3$ , DCPD	SrHA	m	85
$\text{SrCl}_2$	l	$\alpha$ -TCP	SrHA	m	93
$\text{Sr}(\text{NO}_3)_2$	l	$\alpha$ -TCP, TTCP	SrHA	m	94

ACP: amorphous calcium phosphate, ASP: amorphous strontium phosphate, DCPA: monetite, DCPD: brushite, DSPA: strontium hydrogen phosphate anhydrous, SrACP: strontium-substituted ACP, Sr- $\alpha$ -TCP: strontium-substituted  $\alpha$ -TCP, SrCDHA: Sr containing Ca-deficient HA, TTCP: tetra-calcium phosphate. <sup>a</sup> Liquid component did contain polymeric additives. <sup>b</sup> Whiskers.



characterisation of that cement was published.<sup>91</sup> SrCO<sub>3</sub> was also successfully used to gradually replace CaO in a DCPD/CaO cement, resulting in the formation of a SrHA matrix *via* several intermediate steps.<sup>88</sup> However, the Sr/(Ca + Sr) ratio that can be achieved by substituting a Ca phase by its Sr analogue is limited to the fraction the respective Ca phase contributes to the total precursor. Therefore, and despite the elaborate synthesis mostly required to obtain Sr-substituted CaP phases which are often not commercially available, such phases can easily be used to introduce varying amounts of Sr into CPC. For example, the cement forming reaction based on the hydrolysis of  $\alpha$ -TCP into HA upon mixing with aqueous Na<sub>2</sub>HPO<sub>4</sub> results in SrHA when  $\alpha$ -TCP is substituted by Sr- $\alpha$ -TCP although a reduced reactivity was found for Sr- $\alpha$ -TCP synthesised at high temperatures.<sup>73</sup> In a more complex system based on ACP and DCPD, Sr-substituted ACP (SrACP) prepared by gradual replacement of Ca(NO<sub>3</sub>)<sub>2</sub> by Sr(NO<sub>3</sub>)<sub>2</sub> during ACP precipitation from an aqueous solution allows the preparation of cements setting into SrHA.<sup>89</sup> Similarly, SrACP prepared from DCPA, calcium hydroxide and SrCO<sub>3</sub> *via* a dry mechanochemical approach was employed as a Sr source to obtain cements with Sr/(Sr + Ca) ratios between 0.025 and 0.2. Although SrCO<sub>3</sub> residues were detected in the set cement at early time points, ongoing hydration led to a decrease in SrCO<sub>3</sub> content and substitution of Sr<sup>2+</sup>-ions in the apatite matrix.<sup>90</sup>

Based on a cement liquid containing various concentrations of SrCl<sub>2</sub>, cements setting into monophasic Sr-containing apatite can be prepared from  $\alpha$ -TCP<sup>93</sup> and  $\alpha$ -TCP/gelatin<sup>75</sup> as well as DCPA/CaCO<sub>3</sub> precursor powders.<sup>85</sup> Due to the solubility of SrCl<sub>2</sub> and the limited liquid to powder ratio (l/p) a maximum of 6 wt% strontium content could be achieved by the second method. During immersion in water, this cement was shown to release around 80% of the contained strontium within 21 days, indicating a higher solubility of the poorly crystalline, Sr-substituted apatite cement matrix formed during setting.<sup>85</sup> Similarly, when strontium nitrate was mixed into the liquid phase of an  $\alpha$ -TCP/TTCP cement the resulting SrHA with up to 4.3 wt% Sr released more Sr than Ca.<sup>94</sup>

## 2.2 Brushite-forming cements

Most brushite forming cement formulations comprising strontium are based on the synthesis of Sr-substituted precursor phases (Table 4). One approach is to synthesise Sr-substituted TCP as reactive species. Alkhraisat *et al.*<sup>74</sup> prepared Sr- $\beta$ -TCP by sintering CaHPO<sub>4</sub> with mixtures of CaCO<sub>3</sub> and SrCO<sub>3</sub>, thus obtaining Sr- $\beta$ -TCP with molar Sr/(Sr + Ca) ratios between 0 and 0.33. These were used in a hydraulically setting cement upon mixing with an equimolar amount of MCPM. Lattice parameters of the setting product brushite indicated a substitution of Ca<sup>2+</sup> by Sr<sup>2+</sup> ions in the set cement. After a small initial burst, a constant release of Sr-ions from the cements was found during immersion in water, releasing up to 2.3 mg strontium per g cement within 15 days. However, no impact of the Sr-modification on the mechanical properties was found. Another Sr-substituted CPC setting to brushite based on  $\alpha$ -TCP was described by Pina *et al.*<sup>95,96</sup> Firstly, Sr- $\beta$ -TCP was synthesised *via*

Table 4 Selected Sr-containing cement formulations setting into brushite

Sr-source	Solid/liquid	Precursor phases	Setting products	Ref.
Sr- $\beta$ -TCP	s	Sr- $\beta$ -TCP, MCP	Sr-brushite	74
Sr- $\alpha$ -TCP	s	Sr- $\alpha$ -TCP <sup>a</sup>	Sr-brushite	95 and 96
SrCl <sub>2</sub>	s	$\beta$ -TCP, MCP, SPP, SrCl <sub>2</sub>	Sr-brushite	56

MCPM: calcium dihydrogen phosphate (monohydrate), SPP: sodium pyrophosphate, Sr- $\alpha$ -TCP: strontium-substituted  $\alpha$ -TCP, Sr- $\beta$ -TCP: Sr-substituted  $\beta$ -TCP. <sup>a</sup> Cement also contained poly ethylene glycol (PEG) or hydroxyl propyl methylcellulose (HPMC).

aqueous precipitation from calcium nitrate, diammonium hydrogen phosphate and strontium with a (Ca + Sr)/P molar ratio of 1.5, allowing the precipitation of TCP. The precipitate was subsequently treated thermally at 1500 °C to obtain Sr- $\alpha$ -TCP. To accelerate setting, an aqueous solution of citric acid was used as cement liquid, and poly(ethylene glycol) or hydroxyl propyl methylcellulose was added to enhance washout resistance of the cement paste. The cement set into brushite with Sr<sup>2+</sup> ions substituted into the crystal structure. In contrast to the cement described by Alkhraisat *et al.*, this cement exhibited an increased compressive strength compared to a reference material prepared from Sr-free  $\alpha$ -TCP.

Based on the addition of Sr *via* the liquid phase, another cement that sets into Sr-brushite is based on the reaction of  $\beta$ -TCP and MCP in the presence of up to 10 wt% SrCl<sub>2</sub> and traces of sodium pyrophosphate.<sup>56</sup> In this system high Sr content led to a slight decrease in the diametral tensile strength of the set cements.

## 3. Properties of SrCPC

### 3.1 Cement setting and injectability

Setting of CPC has been demonstrated to be fundamentally altered by the presence of Sr<sup>2+</sup> ions by Guo *et al.* when SrHPO<sub>4</sub> was added to a TTCP/DCPA system. Hydration was retarded significantly, which was attributed to the higher degree of supersaturation that is required to yield Sr-containing apatite crystals. Furthermore, the transformation rate into HA or SrHA, respectively, was reduced in the presence of Sr albeit this could be partially compensated by higher phosphoric acid content in the cement liquid.<sup>84</sup> Cements comprising Sr phases as reactants to form SrHA tend to have prolonged setting times if SrCO<sub>3</sub> or DSPA is used as a Sr source.<sup>81,83,86</sup> Sr substitution in ACP, on the contrary, induced faster setting in SrACP/DCPA systems.<sup>90</sup> The setting time of ACP/DCPD cements was reported to significantly increase with the addition of SrCO<sub>3</sub> (with at the same time decreasing viscosity of the cement paste).<sup>87</sup> Hydrolysis of  $\alpha$ -TCP into CDHA was shown to be decreased in the presence of Sr-containing solution.<sup>97</sup> The addition of SrCO<sub>3</sub> into  $\alpha$ -TCP based cements merely affected the setting time, which could be due to the fact that SrCO<sub>3</sub> did not participate in the setting reaction.<sup>86</sup> Sr substituted TCP, whether in the form of  $\alpha$ -TCP setting to SrHA<sup>73</sup> or  $\alpha$ - or  $\beta$ -TCP in brushite forming cements,<sup>74</sup> was shown to slow down the cement setting,



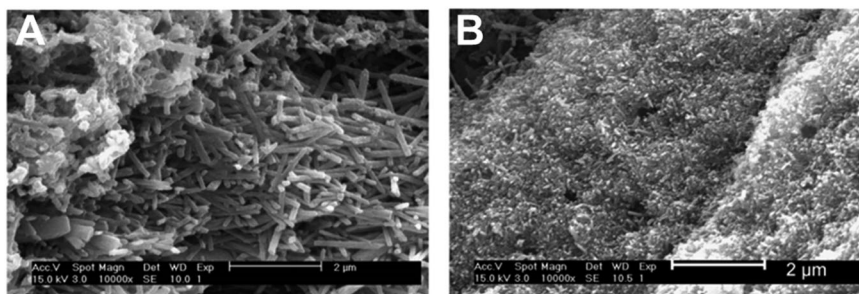


Fig. 4 SEM micrographs of set samples of Sr-free (A) and Sr-containing (B) CPC derived from an ACP/DCPD and ACP/SrCO<sub>3</sub>/DCPD precursor, respectively. Reprinted from X. Wang, *et al.*, Influence of a novel radiopacifier on the properties of an injectable calcium phosphate cement, *Acta Biomater.*, 2007, **3**(5), 757–763, with permission from Elsevier.

although Pina *et al.* stated that lattice distortion caused by Sr substitution enhanced TCP reactivity and did not hinder the conversion of brushite into apatite.<sup>95</sup>

In most cases, the retarding effect of Sr on the nucleation and crystal growth of apatite results in the formation of much smaller crystals during cement setting.<sup>81,86,87,90</sup> This is illustrated in Fig. 4, where smaller crystals are visible after the setting of an Sr-containing, ACP/DCPD-based cement comprising SrCO<sub>3</sub> as Sr source. However, some studies either reported no visible differences in the microstructure of the set cements<sup>83</sup> or at least partially attributed differences in the crystal size to variations in milling parameters during precursor preparation.<sup>86</sup>

Since bone defect treatment *via* minimally invasive surgical techniques (*e.g.* in spinal surgery or in order to reinforce osteoporotic bones) has gained considerable attention over the last few years, controlled injectability of both apatite and brushite cements is of interest.<sup>98,99</sup> Besides powder-to-liquid-ratio, particle size distribution (controlled by precursor milling time) and organic additives like poly(ethylene glycol) or methylcellulose, ion substitution also affects cement setting and thus injectability.<sup>96</sup> By enhancing the reactivity of  $\alpha$ -TCP strontium shortens the setting time and thus impairs the injectability of brushite-forming cements based on Sr- $\alpha$ -TCP.<sup>96</sup> In contrast, SrCO<sub>3</sub>-laden apatite cements were found to gain injectability with increasing Sr content due to a decrease in the viscosity of the cement paste.<sup>87</sup> However, since injectability is also a function of precursor particle size (and size distribution) no general conclusions can be drawn when comparing different studies.

### 3.2 Degradation and strontium-ion release

Degradation of CPC describes the physico-chemical dissolution of the set cements in aqueous environments and is usually quantified by the release of Ca<sup>2+</sup> and phosphate ions. Obviously, this process is controlled by the solubility of the cement components (Table 2), but also by parameters like porosity, surface roughness and area as well as material fragmentation. In general, brushite degrades much faster than hydroxyapatite. Depending on the experimental setup, precipitation of CaP crystals from Ca and phosphate containing immersion liquids can even lead to a virtually negative degradation rate, making it difficult to compare the effects of Sr modification on degradation in different studies. There is, however, general consensus that

lattice expansion induced by the larger Sr<sup>2+</sup> ions upon integration in the apatite lattice increases solubility, and consequently higher degradation was demonstrated for SrHA and SrHA forming cements.<sup>54,55,93,100</sup>

There are two mechanisms that can contribute to the release of Sr<sup>2+</sup> from a SrCPC. One is the diffusion of Sr<sup>2+</sup> from soluble phases in the cement matrix *via* micropores in the cement caused by the cement liquid, and the second is the physico-chemical dissolution of the cement matrix itself. During immersion in large volumes of Ca<sup>2+</sup> and Sr<sup>2+</sup> free buffer solution (*sink* conditions), Sr modification by the addition of solid SrCO<sub>3</sub> led to a reduced Ca<sup>2+</sup> release in a system that set into a SrCO<sub>3</sub>-containing, biphasic HA matrix.<sup>85</sup> Interestingly, Sr introduction into the same cement system *via* the liquid phase, resulting in a monophasic SrHA matrix, had the opposite effect on Ca<sup>2+</sup> release and the Ca<sup>2+</sup> release increased compared to the Sr-free control cement. Sr<sup>2+</sup>, on the other hand, was released to a greater extent from the biphasic cement containing SrCO<sub>3</sub> clusters, where ~80% of the initially contained Sr was released within 21 days of immersion in water.<sup>85</sup> The latter effect was also found in an  $\alpha$ -TCP-based cement system, where cumulative Sr<sup>2+</sup> release from a set biphasic HA/SrCO<sub>3</sub> bulk cement did account for only 1.5% of the initially added Sr but exceeded the release from a monophasic SrHA over 21 days in salt buffer solution.<sup>85,86</sup> It can be hypothesized that this difference was due to Sr<sup>2+</sup> release *via* dissolution of SrCO<sub>3</sub> crystals on the one hand and either diffusion-controlled or degradation-associated Sr release on the other. In a diffusion-controlled system, a depletion of Sr at the sample surface should occur, and leaching of Sr<sup>2+</sup> from the sample surface was shown for monophasic SrHA cements.<sup>85,86</sup> In SrCO<sub>3</sub>/HA cements, a decrease of Sr-concentration at the surface of samples immersed in liquid for 35 days was also present and was traceable even further into the bulk cement, suggesting that diffusion can be a limiting factor also in biphasic cements.<sup>86</sup> For monophasic SrHA cements prepared using a Sr(NO<sub>3</sub>)<sub>2</sub> solution, Leroux *et al.* proposed a two-stage degradation mechanism, comprising an initial phase where Sr<sup>2+</sup> release exceeds Ca<sup>2+</sup> release due to the dissolution of strontium nitrate traces in the set cement, followed by the congruent release of Sr<sup>2+</sup> and Ca<sup>2+</sup> according to the ratio contained in the cement during bulk dissolution. Still, only 2 and 4 wt% of the contained Ca and Sr, respectively, were released within one month.<sup>94</sup>



Although ion release and degradation can more easily be studied in ion-free buffer solutions, more complex immersion media are required to obtain results comparable to the *in vitro* (or *in vivo*) situation. Typical for  $\alpha$ -TCP-based materials is a slight acidification and a depletion of  $\text{Ca}^{2+}$  concentration in the immersion medium that arises from the presence of CDHA and the progressive formation of apatite crystals.<sup>101,102</sup> Since material-mediated Ca depletion can have significant impact on the cellular response,<sup>103,104</sup> it is important to note that Sr modification can, in some systems, alter this effect. For an  $\alpha$ -TCP/DCPA/ $\text{CaCO}_3$ /HA-based apatite cement immersed in alpha minimum essential cell culture medium ( $\alpha$ MEM)  $\text{Ca}^{2+}$  concentration was shown to drop from 1.8 mM to  $\sim 0.5$  mM and remain low over 21 days despite a regular medium change. Strontium modification, either by addition of  $\text{SrCO}_3$  crystals or substitution of  $\text{CaCO}_3$  by  $\text{SrCO}_3$  in the precursor, resulted in a significant reduction of Ca depletion. Sr was, at the same time, released in higher doses from monophasic SrHA forming cements, resulting in concentrations of 0.05–0.1 mM compared to 0.025–0.05 mM for  $\text{SrCO}_3$ /HA biphasic cements.<sup>105</sup>

For brushite-forming cements prepared from Sr substituted  $\beta$ -TCP a high, zero-order release of Sr was found during immersion in water. Up to 2.3 mg  $\text{g}^{-1}$  Sr was released, and the kinetics of Sr release resembled the one measured for Ca, indicating that Sr was released by bulk erosion.<sup>74</sup> In an approach where Sr was introduced into the cement *via* the liquid phase an increase in solubility with increasing Sr-content was described.<sup>56</sup>

It is obvious that degradation and thus  $\text{Sr}^{2+}$  release differ from any laboratory setup as soon as a SrCPC is implanted into a tissue defect. Besides the complexity of body fluids compared to any of the buffer solutions described above, diffusion processes within the tissue as well as (cell-driven) active ion transport occur. Still several *in vivo* studies demonstrated an increased degradation of SrCPC compared to the respective Sr-free control groups and thus confirmed the above findings.<sup>106,107</sup> However,  $\text{Sr}^{2+}$  release did in no case result in an increase of serum

strontium levels to concentrations comparable to strontium ranelate treatment, suggesting that the released  $\text{Sr}^{2+}$  is accumulated in the surrounding bone.<sup>91</sup>

### 3.3 Mechanical characteristics of SrCPC

As all inorganic cements and ceramics, CPC in general exhibit brittle fracture behaviour. Compared to natural bone (*cancellous bone*: 5–15 MPa and 0.26–0.90 GPa, *cortical bone*: 133–193 MPa and 17–25 GPa compressive strength and compression modulus, respectively)<sup>1,108</sup> most CPC possess poor mechanical strength and thus are mainly intended to be used in non-load-bearing situations. Brushite cements tend to have a lower compressive strength (10–60 MPa)<sup>109–111</sup> than hydroxyapatite forming ones (up to 75 MPa),<sup>109,112</sup> depending mainly on the phase composition of the set cement as well as on the respective porosity.<sup>112</sup>

In SrCPC, contradicting results have been found regarding mechanical characteristics that are summarized in Table 5. It is important to note that the different preparation routes and properties of the set samples, such as porosity, do not allow direct comparison of the mechanical characteristics. In general, the strength of the cements increases within the first days to weeks as hydration proceeds. In most cements setting into monophasic SrHA, strontium modification either increased the compressive strength<sup>83,86,90</sup> or had no significant effect on the mechanical characteristics.<sup>81</sup> Nevertheless, one study reported a decrease in the compressive strength of Sr- $\alpha$ -TCP cements compared to Sr-free control.<sup>73</sup> This was attributed to a decreased reactivity of the precursor that was derived from the high sintering temperature during Sr- $\alpha$ -TCP synthesis which caused a larger crystal size and therefore slower dissolution speed which was also obvious from the prolonged setting time. To explain the higher strength of most SrHA cements based on multi-component precursors, Yu *et al.* suggest an increase in Ca 2p and P 2p binding strength in the Sr-substituted apatite lattice as measured by XPS.<sup>90</sup> Other parameters in discussion are the occurrence of crystal defects and the inhibitory effect of Sr on the

Table 5 Properties of set SrCPC

Setting product	Porosity [vol%]	Compressive strength <sup>a</sup> [MPa]	Initial setting time [min]	Final setting time [min]	Ref.
SrHA (m)	n/a	$\sim 66$ (+)	(+)	(+)	83
SrHA (m)	52 (–)	n/a	n/a	n/a	85
SrHA (m)	< 5	12–13 (/)	6–34 (–)	13–82 (–)	81
SrHA (m)	n/a	$\sim 10$ (–)	(–)	(–)	73
SrHA (m)	56–58	63–74 (+)	21–28 (+)	37–52 (+)	90
SrHA (m)	30–42 (+)	$\sim 53$ (+)	$\sim 13$ min (+)	$\sim 33$ min (+)	86
SrHA (m)	n/a	10–20 (+)	11–17 (+)	17–21 (+)	88
SrCDHA (m)	38–47 (+)	4–13 (+/–)	4–20 (+)	9–50 (+)	75
HA/ $\text{SrCO}_3$ (b)	(+)	40 (+)	$\sim 30$ –40 (+)	$\sim 45$ –55 (+)	87
HA/ $\text{SrCO}_3$ (b)	49 (–)	n/a	n/a	n/a	85
HA/ $\text{SrCO}_3$ (b)	26–40 (–)	$\sim 15$ (–)	$\sim 7$ min (/)	$\sim 26$ min (/)	86
Sr-brushite (m)	$\sim 50$ (/)	$\sim 5$ –6 (/)	2–5 (+)	n/a	74
Sr-brushite (m)	n/a	$\sim 22$ (+)	3–7 (+)	n/a	95

(+) increased, (/) unaffected, (–) decreased (against Sr free reference). m = monophasic, b = biphasic cement matrix after setting. <sup>a</sup> For series with different Sr/(Sr + Ca) ratios the maximum strength is shown.



apatite depositing rate.<sup>83</sup> In their TTCP/DCPA/DSPA cement system, Guo *et al.* described a non-linear influence of Sr content on the mechanical properties: while an optimum composition was found at a Sr/(Sr + Ca) ratio of 0.05 with an increase of compressive strength up to 66 MPa compared to ~50 MPa for the Sr-free reference cement, higher Sr content resulted in a reduced compressive strength.<sup>83,84</sup> A comparable optimum was described for DCPD-based cements where SrCO<sub>3</sub> replaced the second precursor component, CaO.<sup>88</sup> Here, up to 1.5% molar fraction of SrCO<sub>3</sub> increased the compressive strength of the set cement from ~7 to ~20 MPa. Again, higher substitution resulted in decreased strength values. In contrast Kuang *et al.* found no effect on the mechanical properties for the same cement system with partial or complete substitution of DCPA by DSPA (Sr/(Sr + Ca) = 0–0.2), although the overall compressive strength was significantly lower (~12 MPa after 28 days).<sup>81</sup> Still, in this cement a polymer component had been added to the cement liquid which might have interfered with the setting process. Full replacement of DCPA by DSPA and CaCO<sub>3</sub> by SrCO<sub>3</sub>, on the other hand, reduced the compressive strength of  $\alpha$ -TCP/DCPA/CaCO<sub>3</sub>/HA cements.<sup>91</sup> In contrast, in another monophasic system, up to 8.37 wt% strontium in SrHA derived from an  $\alpha$ -TCP/DCPA/HA/CaCO<sub>3</sub> precursor by substitution of CaCO<sub>3</sub> by SrCO<sub>3</sub> increased the compressive strength significantly from 32 to 53 MPa.<sup>86</sup> In a SrACP/DCPD cement higher compressive strength values were found for Sr/(Sr + Ca) ratios between 0.025 and 0.2 (up to 75 MPa after 10 days) compared to Sr-free reference cement. Although residues of SrCO<sub>3</sub> were present in the cement at early time points, hydration was shown to lead to a decrease in SrCO<sub>3</sub> content and the integration of Sr in the apatite matrix.<sup>90</sup> Sr modification *via* the cement liquid also results in the formation of SrHA; however, only modest impact on the mechanical properties was described by Panzavolta *et al.*, where 1 wt% substitution increased compressive strength, whilst higher substitution resulted in decreased strength.<sup>75</sup>

In cement systems setting into HA–SrCO<sub>3</sub> composite matrices, a decrease of mechanical strength compared to unmodified cement control samples was reported. This was explained by the low binding strength of SrCO<sub>3</sub> residues and the surrounding apatite matrix and the role of these crystals as crack initiators under compression loading.<sup>86</sup> Wang *et al.* postulated a small amount of Sr substitution into the HA lattice from intermixed SrCO<sub>3</sub> particles and thus a small increase in compressive strength, whilst at higher SrCO<sub>3</sub> loading ratios compressive strength also decreased.<sup>87</sup> In contrast to residual SrCO<sub>3</sub> clusters with poorly defined morphology, addition of SrHA whiskers was shown to significantly increase the strength of set cements at concentrations of 2.5 and 5 wt%. This result was attributed to the entanglement of the elongated whiskers, although at higher contents (10 wt%) a decrease of compressive strength was found.<sup>92</sup>

In brushite forming cements based on Sr- $\beta$ -TCP the presence of Sr did merely affect the compressive strength, as well as porosity, although a tendency to set into monetite instead of brushite was found at Sr contents up to 20 at%.<sup>74</sup> In contrast, Pina *et al.* found elevated compressive strength in Sr- $\alpha$ -TCP

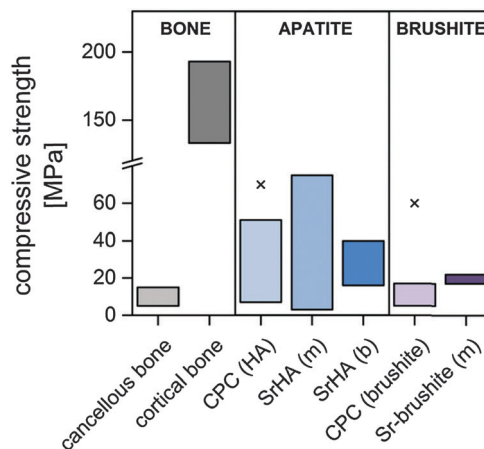


Fig. 5 Comparison of compressive strength values of natural bone, different SrCPC (m: mono- and b: biphasic setting product) and their respective Sr-free reference materials. "x" indicates the highest strength values of other HA and brushite cements described in the literature.<sup>1,75–75,81,83,85–88,90,95,108</sup>

based cements, and attributed this to the reduced conversion of the precursor in the mechanically weaker setting product brushite. However, the high solubility of brushite leads to a fast degradation of the cement which resulted in a decrease of strength over time.<sup>95</sup>

In general, the mono- or biphasic nature of the setting product of the respective cement system seems to be a key parameter for the mechanical properties: while the gain in strength caused by crystal lattice deformation associated with Sr substitution can lead to a certain increase in both apatite and brushite cement strength the presence of secondary clusters impairs the mechanical integrity of the set cement (Fig. 5).

### 3.4 Radiopacity

Apart from the therapeutic benefits of Sr modification and possible improvement of mechanical properties, another rationale to introduce Sr-phases into CaP bone cements is their increasing effect on the adsorption of X-rays. This is of interest since high radiopacity is required to monitor the positioning of injectable cements during surgery and follow-up imaging. Various species such as SrCl<sub>2</sub>, SrCO<sub>3</sub>, SrBr<sub>2</sub>, and SrF<sub>2</sub> have been evaluated to modify CPC<sup>86–88,90,113,114</sup> or calcium–aluminium–phosphate cements<sup>115</sup> for that purpose. In most cases an increase of radiopacity with Sr content was shown. For example, Wang *et al.* demonstrated that 4–20% Sr substitution allows one to distinguish SrCPC from bone samples with the same thickness in radiographic images (Fig. 6).<sup>87</sup> When comparing cements setting to SrHA and SrCO<sub>3</sub>/HA the latter showed less increase of radiopacity at similar Sr contents which was attributed to the low resolution of the radiographic imaging that could not correctly represent small local contrast variations as to be expected in biphasic matrices.<sup>86</sup>

### 3.5 Biological properties based on cell culture studies

Only a few data have been published on the *in vitro* characteristics of Sr-modified CPC. In general, Sr modification does not



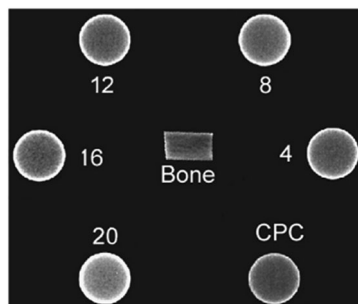


Fig. 6 Radiograph of cortical bone and CPC samples (5 mm thickness) with 4–20% Sr content. Reprinted from X. Wang, *et al.*, Influence of a novel radiopacifier on the properties of an injectable calcium phosphate cement, *Acta Biomater.*, 2007, **3**(5), 757–763, with permission from Elsevier.

induce cytotoxicity. In cell cultures treated with extracts of SrHA,<sup>81,86</sup> biphasic HA/SrCO<sub>3</sub><sup>86</sup> and SrHA-whisker reinforced cements,<sup>92</sup> a positive effect of the Sr doping and/or the Sr<sup>2+</sup> released from the cements was found on cell proliferation and osteogenic differentiation. It was further stated that the decreased tendency of SrCPC to cause medium Ca<sup>2+</sup> depletion as well as a smaller variation in the pH during SrCPC setting may contribute to these findings.<sup>86</sup> Only one study described a slight cytotoxic effect of highly concentrated SrHA extracts on the murine connective tissue cell line L929.<sup>83</sup> Table 6 shows an overview of the *in vitro* effects of SrCPC. For example, Tadier *et al.* found a higher proliferation rate of osteoprogenitor cells on biphasic SrCO<sub>3</sub>/HA compared to monophasic SrHA cements that were derived from a DCPD/CaCO<sub>3</sub>/SrCO<sub>3</sub> precursor. However, at an mRNA level there was no difference in osteogenic differentiation.<sup>85</sup> In contrast two different routes of Sr modification based on an  $\alpha$ -TCP precursor, setting into SrHA and SrCO<sub>3</sub>/HA, respectively, were both shown to stimulate proliferation and osteogenic differentiation of primary human mesenchymal stem cells.<sup>105</sup> Interestingly, proliferation was generally more enhanced in samples kept under basal cell culture conditions, whilst cells cultured in medium supplemented with osteogenic additives showed higher proliferation on monophasic SrHA cement matrices. This indicates different susceptibilities of cells towards Sr depending on their stage of differentiation. No cytotoxicity (using MG63 osteoblast-like cells in an indirect culture setup) but an enhanced proliferation and osteogenic differentiation in a direct setup using MC3T3-E1 murine pre-osteoblasts were found for TTCP/DCPA/DSPA based SrHA cements. A comparable cell morphology was found on SrCPC and Sr free controls.<sup>107</sup> Similarly, enhanced proliferation and osteogenic differentiation were observed for MG63 osteoblasts cultured on  $\alpha$ -TCP/gelatine/DCPD cements where Sr was introduced *via* the liquid phase.<sup>75</sup> Since the stimulatory effect of Sr modification was found in indirect cell culture setups as well as in direct contact cultures, it can be hypothesised that different aspects contribute to the positive cell reaction: release of Sr<sup>2+</sup> from the cement, altered ionic interaction of the cement and the cell culture medium, resulting in *e.g.* reduced Ca<sup>2+</sup> depletion and reduced acidification of the medium.<sup>85,105</sup>

Table 6 Properties of selected SrCPC in cell culture experiment

Setting product(s)	Sr content	Cell type	Sr <sup>2+</sup> concentration in cell culture medium	Cell proliferation	Osteogenic differentiation	Cell culture setup: extract (E), direct (D) or powdered cement (P)	Ref.
SrCDHA (m)	0–5 wt%	MG63	n/a	Increased with Sr content	Increased <sup>a</sup>	D	75
SrHA (m)	0–6 wt%	hBMSC	<10 ppm	No effect	No effect <sup>a</sup>	D	85
HA/SrCO <sub>3</sub> (b)	0–8 wt%	hBMSC	<200 ppm	Increased with Sr	No effect <sup>a</sup>	D	85
SrHA (m)	0–10% Sr/(Sr + Ca)	L929	n/a	No effect	n/a	E	83
SrHA (m)	0–8.37 wt%	hBMSC	0.025–0.125 mM	Increased <sup>a</sup>	Increased <sup>a</sup>	E, D	105
HA/SrCO <sub>3</sub> (b)	0–5.4 wt%	hBMSC	0.05–0.14 mM	Increased <sup>a</sup>	Increased <sup>a</sup>	E, D	105
SrHA (m)	0–33% Sr/(Sr + Ca)	MG63	n/a	Increased <sup>a</sup>	Increased <sup>a</sup>	E, D	81 and 107
HA/SrHA-whisker (b)		MC3T3 E1					
		MG63	0.171–1.39 mM	Increased	n/a	E	92
Sr-brushite (m)	0–33% Sr/(Sr + Ca)	hFOB1.19	n/a (10–40 mg ml <sup>-1</sup> measured in water)	Unaffected <sup>a</sup>	n/a	D	74
ZnSr-brushite	2.1 mol%	MC3T3-E1	n/a	Increased	Unaffected <sup>a</sup>	E, P	117

MG62: human osteoblast-like cell line; hBMSC: human bone marrow-derived stromal/stem cells; L929: murine connective tissue cell line; hFOB1.19: human fetal osteoblast cell line; MC3T3-E1: murine pre-osteoblast cell line. <sup>a</sup> Against Sr free reference.



In Sr containing brushite cements prepared from Sr- $\beta$ -TCP and MCPM, no distinct influence of Sr content on proliferation and metabolic activity of human osteoblasts was found in a direct setup. The cement released up to 40 ppm (0.56 mM) strontium.<sup>74</sup> However, this release test was performed during immersion in water, and might not correctly represent the situation in cell culture conditions, as it is known that *e.g.* protein content of immersion solutions can limit ion release.<sup>116</sup> In comparison to Zn-doped brushite cements additional doping with Sr was shown to increase the metabolic activity of murine osteoblast precursors.<sup>117</sup> Therefore it can be concluded that the beneficial effects of Sr on both proliferation and differentiation of osteogenic cells were confirmed for SrCPC.

Until today, to the best of our knowledge no data have been published on the osteoclastic resorption of Sr containing CPC *in vitro*. Still, the anti-resorptive effects of Sr incorporated in hydroxyapatite have been demonstrated by Yang *et al.* using mineralised films prepared by precipitation from Ca and Sr containing liquids, that somewhat resemble the characteristics of set CPCs: osteoclastic activity, by means of resorption pit area, was significantly decreased in the presence of different Sr<sup>2+</sup> concentrations while osteoblast proliferation increased in a Sr dose-dependent manner and osteoblast activity remained unaffected.<sup>118</sup>

### 3.6 *In vivo* studies on strontium-containing cements

Although the stimulating effect of systemically administered strontium ranelate on bone formation has been questioned recently,<sup>119</sup> evidence exists that the local release of Sr<sup>2+</sup> into a specific bone defect can support defect healing. One of the first studies using a Sr-containing bone cement *in vivo* was published by Ni *et al.* in 2006, although SrHA was only used as

a filler in a resin-based cement.<sup>120</sup> However, promising results were obtained, with the Sr-laden cement exhibiting an increased bonding strength to bone compared to the Sr-free PMMA cement control group. Positive results for new bone formation at the bone-implant area of SrHA-containing polymeric cements were also described by others.<sup>121–124</sup> Wong *et al.* pointed out that neither inflammatory response nor necrotic reactions or fibrous encapsulation that occurred in the PMMA control group were found in the SrHA-PMMA cement group.<sup>123</sup>

*In vivo* studies focussing on SrCPC of different compositions are summarised in Table 7. In an intramuscular implantation study, Dagang Guo *et al.* demonstrated an increase of material degradation with increasing Sr/(Ca + Sr) ratio using pre-set cylinders prepared from a TTCP/DCPA/DSPA cement with up to 10% strontium substitution.<sup>106</sup> This was confirmed in bone contact when the same material was implanted into a femoral drill hole defect. Furthermore, a tight bond of newly formed bone was found at the interface between the cement and the host bone. Similarly, Kuang *et al.* implanted pre-set TTCP/DCPA/DSPA based SrHA cement cylinders in a metaphyseal drill-hole defect model in rats.<sup>107</sup> Again, enhanced degradation was found for SrHA compared to Sr-free reference after 32 weeks; however, it remains unclear whether this was caused by higher physico-chemical dissolution or cellular resorption. Early endochondral ossification after 2 weeks and higher new bone deposition (4 weeks post operation) indicated an enhanced osteo-regenerative potential of the SrHA cement, although only a small number of animals was used. In another study, it was shown that a SrHA cement accelerated graft healing in anterior cruciate ligament reconstruction using allogenic ligament grafts impregnated with a Sr-containing cement in comparison to Sr-free control.<sup>125</sup>

Table 7 Summary of animal studies on SrCPC

Cement type	Animal model	Type of defect	Results	Ref.
SrHA (pre-set)	8–10 month old New Zealand rabbits	Intramuscular ( <i>m. sacrospinalis</i> ) Drill hole (2.2 mm)	Cement degradation increased with the Sr/(Sr + Ca) molar ratio during intramuscular implantation for 24 weeks, reaching approx. 50% in the 10% substituted group. After 4–24 weeks degradation increased with Sr-content (up to 10%). Good integration of 10% SrCPC was found. Sr was located in the newly mineralised surrounding tissue.	106
SrHA (pre-set)	3 month old Sprague-Dawley rats	Drill hole (2.5 × 4 mm) metaphyseal	Enhanced endochondral ossification was found in the 10% Sr-CPC group after 2 weeks, whilst after 4–16 weeks post-operation progressive osteo-conduction was found in both groups.	107
n/a ( $\alpha$ -TCP based)	10 week old Sprague-Dawley rats (♀) 2 months after OVX, OPen	Drill hole (2 mm) metaphyseal	After 1, 3 and 6 months, SrCPC exhibited increased fragmentation. Faster osseointegration and increased new bone formation were found in the SrCPC group compared to Sr-free reference. Fragmentation promoted implant integration.	91
SrHA	10 week old Sprague-Dawley rats (♀), 12 weeks after OVX, D, OPen	Metaphyseal, 4 mm wedge-shaped, bridged with an osteosynthesis plate	After 6 weeks a significant increase of new bone formation in the SrCPC group against CPC and empty defect was found. Upregulation of bone-specific markers bone-morphogenic protein-2, osteocalcin, osteoprotegerin and alkaline phosphatase; increased collagen formation. Sr <sup>2+</sup> was found in the newly formed bone tissue around the implant.	127
ZnSr-brushite	Pig (♂, n = 2)	Drill hole (3.5 × 10 mm <sup>3</sup> ) trabecular	After 1 and 2 months the highest new bone formation with the most pronounced organisation level was found in the SrZn-group compared to Sr-free ZnCPC and CDHA cement control (commercial). Less osteoclasts were found around SrZnCPC.	117

OVX: ovariectomized; D: animals received calcium and phosphate deficient diet; OPen: animals had confirmed pre-implantation osteopenic bone status.



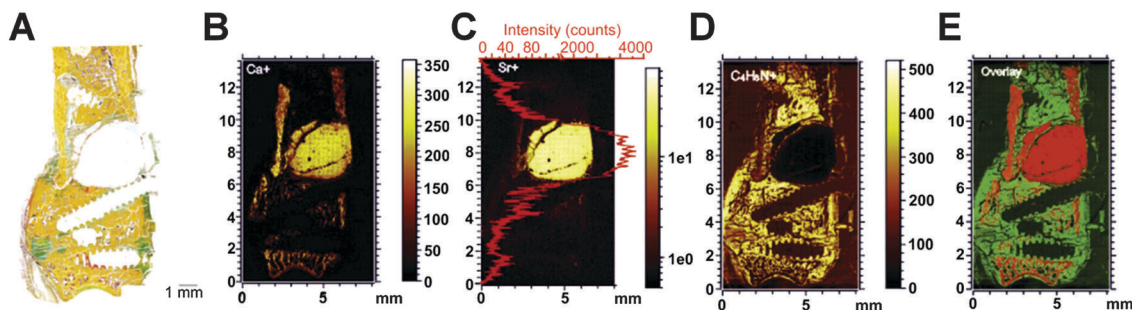


Fig. 7 Histological overview of the implant region where a Sr-modified CPC was implanted into a metaphyseal critical-size defect in the femora of ovariectomized rats (A) and mass distribution of  $\text{Ca}^+$  (B),  $\text{Sr}^+$  (C) and  $\text{C}_4\text{H}_8\text{N}^+$  (D) and overlay of  $\text{Ca}^+$ ,  $\text{Sr}^+$  and  $\text{C}_4\text{H}_8\text{N}^+$  (E) as measured by ToF-SIMS. Reprinted from U. Thormann, *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, with permission from Elsevier.

To study the effects of Sr-modified CaP intended to positively influence the bone metabolism in osteoporotic bone *in vivo*, the key issue is to select an appropriate animal model that resembles the human osteoporotic fracture situation.<sup>126</sup> Baier *et al.* implanted an  $\alpha$ -TCP/SDPA/SrCO<sub>3</sub>/HA cement in a metaphyseal drill hole defect in ovariectomized rats in comparison to a Sr free reference cement.<sup>91</sup> It is interesting to note that the occurrence of discontinuities within the cement appeared as a key for cement integration into the bone tissue, since new bone formation was found at these sites and was even enhanced in the case of Sr-laden cement. Moreover, BMD measurements revealed a local increase of bone in the cement-treated metaphyseal region, suggesting that Sr released from the cements led to a local bone-anabolic effect. This is supported by a recent study, where ToF-SIMS imaging was used to visualize Sr-distribution in histological sections of the metaphyseal region of rat femora where a SrHA cement based on  $\alpha$ -TCP, DCPA, SrCO<sub>3</sub> and HA had been implanted to bridge a wedge-shaped critical size defect resembling a typical osteoporotic fracture (Fig. 7).<sup>127</sup> Furthermore, histomorphometric analysis of bone formation as well as immunohistochemical analysis of bone-related markers confirmed an increased bone formation and remodelling activity in the Sr-HA group.

Only one study focussing on the *in vivo* properties of Sr-containing brushite cement has been published so far: in a trabecular bone defect in pigs Pina *et al.* demonstrated positive effects of Sr co-doping of a Zn-containing brushite cement. Sr doping was found to reduce the osteoclast density at the bone-implant interface and to increase new bone formation.<sup>117</sup>

Although data on the *in vivo* effects of SrCPC are sparse, published data strongly suggest that Sr doping of CPC positively influences local bone regeneration. In particular animal studies in osteoporotic models revealed promising results towards the utilisation of SrCPC in the clinical treatment of osteoporosis related fractures.

## 4. Conclusions

A variety of approaches towards strontium-substituted calcium phosphate bone cements have been published in the last few years.

Most of them are based on the use of Sr-containing phases that are intermixed with the conventional precursor powder or substitute CaP phases therein, whilst others use the cement liquid to introduce Sr<sup>2+</sup> into the cement. The resulting cements can be categorised into those that set into mono- or multiphasic matrices that are characterised by a homogeneous Sr distribution or contain Sr rich clusters, respectively. In general, Sr substituted CPC are characterised by an enhanced degradability both under laboratory conditions and *in vivo*. Given the differences in the phase composition and porosity of the cements no general conclusion on the effects of Sr modification on the mechanical properties of the set cements can be drawn, but an increased strength has been found in many compositions setting into monophasic SrHA, whereas multi-phasic cements tend to have lower strength. The stimulatory effect of Sr<sup>2+</sup> released from the cements has clearly been shown *in vitro*, although the use of various cell types that may have different susceptibilities to Sr<sup>2+</sup> concentrations, varying experimental setups and cement characteristics (*e.g.* porosity) prohibit general conclusions on the optimal degree of substitution. There remains a need to study the influence of Sr modification on bone resorbing cells *in vitro*. However, evidence exists from a few *in vivo* studies that the potential of Sr<sup>2+</sup> to stimulate bone formation can be used to locally enhance new bone formation without systemic side effects. Therefore, strontium modified calcium phosphate bone cements are promising materials that could help to improve clinical outcome in the regeneration of bone defects, in particular in the case of diseased, *e.g.* osteoporotic, bone.

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