



Full length article

## Strength reliability and *in vitro* degradation of three-dimensional powder printed strontium-substituted magnesium phosphate scaffolds



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### ABSTRACT

Strontium ions ( $\text{Sr}^{2+}$ ) are known to prevent osteoporosis and also encourage bone formation. Such twin requirements have motivated researchers to develop Sr-substituted biomaterials for orthopaedic applications. The present study demonstrates a new concept of developing Sr-substituted  $\text{Mg}_3(\text{PO}_4)_2$ -based biodegradable scaffolds. In particular, this work reports the fabrication, mechanical properties with an emphasis on strength reliability as well as *in vitro* degradation of highly biodegradable strontium-incorporated magnesium phosphate cements. These implantable scaffolds were fabricated using three-dimensional powder printing, followed by high temperature sintering and/or chemical conversion, a technique adaptable to develop patient-specific implants. A moderate combination of strength properties of 36.7 MPa (compression), 24.2 MPa (bending) and 10.7 MPa (tension) were measured. A reasonably modest Weibull modulus of up to 8.8 was recorded after uniaxial compression or diametral tensile tests on 3D printed scaffolds. A comparison among scaffolds with varying compositions or among sintered or chemically hardened scaffolds reveals that the strength reliability is not compromised in Sr-substituted scaffolds compared to baseline  $\text{Mg}_3(\text{PO}_4)_2$ . The micro-computed tomography analysis reveals the presence of highly interconnected porous architecture in three-dimension with lognormal pore size distribution having median in the range of 17.74–26.29  $\mu\text{m}$  for the investigated scaffolds. The results of extensive *in vitro* ion release study revealed passive degradation with a reduced  $\text{Mg}^{2+}$  release and slow but sustained release of  $\text{Sr}^{2+}$  from strontium-substituted magnesium phosphate scaffolds. Taken together, the present study unequivocally illustrates that the newly designed Sr-substituted magnesium phosphate scaffolds with good strength reliability could be used for biomedical applications requiring consistent  $\text{Sr}^{2+}$ -release, while the scaffold degrades in physiological medium.

### Statement of significance

The study investigates the additive manufacturing of scaffolds based on different strontium-substituted magnesium phosphate bone cements by means of three-dimensional powder printing technique (3DPP). Magnesium phosphates were chosen due to their higher biodegradability compared to calcium phosphates, which is due to both a higher solubility as well as the absence of phase changes (to low soluble hydroxyapatite) *in vivo*. Since strontium ions are known to promote bone formation by stimulating osteoblast growth, we aimed to establish such a highly degradable magnesium phosphate ceramic with an enhanced bioactivity for new bone ingrowth. After post-processing, mechanical strengths of up to 36.7 MPa (compression), 24.2 MPa (bending) and 10.7 MPa (tension) could be achieved. Simultaneously, the failure reliability of those bioceramic implant materials, measured by Weibull modulus calculations, were in the range of 4.3–8.8. Passive dissolution studies *in vitro* proved an ion release of  $\text{Mg}^{2+}$  and  $\text{PO}_4^{3-}$  as well as  $\text{Sr}^{2+}$ , which is fundamental for *in vivo* degradation and a bone growth promoting effect. In our opinion, this work broadens the range of bioceramic bone replacement materials suitable for additive manufacturing processing. The high biodegradability of MPC ceramics together with the anticipated promoting effect on osseointegration opens up the way for a patient-specific treatment with the prospect of a fast and complete healing of bone fractures.

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## 1. Introduction

One of the treatment approaches for healing of critical size bone defects caused by trauma or tumour resection involves the implantation of bioceramics to maintain skeleton functionality. Among the many synthetic calcium phosphate ceramics and cements, farringtonite ( $\text{Mg}_3(\text{PO}_4)_2$ ) and struvite ( $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ ) based on magnesium phosphate chemistry have attracted wider interest due to the fact that biodegradation is enhanced compared to calcium phosphate bone compounds [1,2]. This aspect is decisive as autologous bone is still the gold standard in hard tissue replacement [3–5]. A second advantage of magnesium based bone cements is the lack of phase conversion into low soluble hydroxyapatite (HA;  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ), since  $\text{Mg}^{2+}$  ions are adsorbed on the surface of newly formed HA crystals by competing with and replacing  $\text{Ca}^{2+}$  ions. By blocking the crystallization site, further crystal growth of HA is then inhibited [6,7].

Several studies have already proved that HA can hardly be resorbed under physiological conditions compared to other calcium and magnesium phosphate bone cements [1,2,8,9]. Thus, the restriction of HA formation is favourable for bone substitutes and overall healing of bone defects. The inhibition of HA by incorporation of  $\text{Mg}^{2+}$  and degradation of magnesium-based cements also leads to  $\text{Mg}^{2+}$  ion release into surrounding tissue, serum and circulating blood, whereas bone serves as a storage of  $\text{Mg}^{2+}$  and also helps in regulating ion balance [10,11]. It is worthwhile to mention that the daily uptake (and excretion) of  $\text{Mg}^{2+}$  is in the range of 265 mg/day to 420 mg/day for adults (depending on age and gender) [11,12], and side effects such as hypermagnesemia [12,13] are unlikely to happen as a result of implanted magnesium phosphate ceramics. Recent calculations indicated an  $\text{Mg}^{2+}$  ion release of degrading struvite cement of only 22.8 mg/day [14] and 1.08  $\mu\text{g}/\text{day}$  (for a 1 g implant) [15] and such release can unlikely lead to hypermagnesemia.

Following the degradation of bone substitute implants, new bone formation is essential for the replacement by fully regenerated bone. Several studies have already proved that strontium ions ( $\text{Sr}^{2+}$ ) promote new bone formation due to their chemically similar behaviour compared to  $\text{Ca}^{2+}$  [16–22]. The influence of  $\text{Sr}^{2+}$  is based on a cellular level, affecting both osteoblast and osteoclast cell fate processes [23,24]. In particular, the  $\text{Sr}^{2+}$  ions are reported to promote the proliferation of osteoblast precursor cells and the differentiation into mature osteoblasts [25,26]. Furthermore, strontium induces the activation of calcium sensing receptors within osteoblasts [27,28]. This aspect has two major implications – (i) stimulation of osteoprotegerin production and (ii) release of receptor activator of the nuclear factor  $\kappa\text{B}$  ligand (RANKL), which is responsible for osteoclastogenesis [29]. Osteoprotegerin binds to RANKL, which is expressed by osteoblasts [23]. Therefore, osteoprotegerin reduces the amount of free RANKL for osteoclastogenesis [30] and hence leads to a reduced cellular mediated bone resorption. Apart from that,  $\text{Sr}^{2+}$  exponentiates the effect of  $\text{Ca}^{2+}$  in terms of inducing osteoclast apoptosis, leading to a decreased bone resorption [31].

In addition to designing the formulation for bone reconstructive cements, processing of these materials is an important aspect to facilitate therapeutically successful results. In recent years, numerous additive manufacturing processes were developed for, or adapted to the fabrication of anatomically tailored patient-specific implants. Most important techniques involve stereolithography [32], selective laser sintering [33,34], three-dimensional (3D) plotting and 3D printing [35–45]. The 3D powder printing (3DPP) technology serves as an excellent tool for the fabrication of patient-specific ceramic implants with geometrically complex shape [46]. Computed tomography provides computer aided design (CAD) data of a patients individual defect structure. These

data are digitally converted into a laminated structure that can be printed via a layer-by-layer technique. The use of a reactive binder leads to a hydraulic setting reaction, whereas the powder undergoes a phase transition and forms a solid by crystal growth [47–49]. Apart from this, a polymeric binder or a polymeric powder component can be utilised to act as glue leading to adhesion of powder particles [36,50]. The latter technique includes a post-processing by sintering of the printed samples. During sintering, polymeric additives will be removed and simultaneously powder particles will be sintered and densified. Biocompatible implants for bone replacement manufactured by 3DPP mainly used calcium phosphate cements (CPC) such as brushite, tricalcium phosphate or HA [48,50–53]. These bone cements, however, are either only slowly degradable or they may undergo a phase transformation during *in vivo* into lower soluble phases and therefore are not suitable for a non-permanent implant. Magnesium phosphate cements (MPC) are an adequate alternative due to their biodegradation and mechanical stability. To the best of our knowledge, only Vorndran et al. and Klammert et al. fabricated MPC using 3DPP [39,49] by employing an ammonium phosphate dibasic solution as binder to facilitate a phase conversion from farringtonite to struvite.

In the above perspective, the present work aimed to fabricate degradable freeform implants with enhanced bioactivity by 3DPP of MPC and strontium-substituted MPC (SrMPC) using 3D printing route with degassed ultrapure water as binder. Since the antiosteoporotic effect of strontium strongly depends on the extent of  $\text{Sr}^{2+}$  doping/incorporation [54], studies on such material, i.e. SrMPC need to consider different amounts of  $\text{Sr}^{2+}$  ions introduced to the implantation site. Accordingly, the magnesium phosphate powders ( $\text{Mg}_3(\text{PO}_4)_2$ ) were supplemented with two different  $\text{Sr}^{2+}$  concentrations:  $\text{Mg}_{2.5}\text{Sr}_{0.5}(\text{PO}_4)_2$  and  $\text{Mg}_2\text{Sr}_1(\text{PO}_4)_2$ . Fabrication was accomplished by particle adhesion with cellulose powder addition followed by sintering, leading to mechanically stable three-dimensional parts. In addition, a phase conversion was obtained after sintering by immersion in ammonium phosphate dibasic solution. Apart from the extensive mechanical property measurements including critical analysis of strength reliability, *in vitro* degradation products of MPC were also investigated. An emphasis has been placed to evaluate strength reliability as well as micro-computed analysis of pore architecture and a comparison is made consistently between the sintered and chemically hardened scaffolds with different levels of Sr-substitution.

## 2. Materials and methods

### 2.1. Powder synthesis and 3D powder printing

Magnesium phosphate with three different concentrations for Sr-substitution was used,  $\text{Mg}_3(\text{PO}_4)_2$ ,  $\text{Mg}_{2.5}\text{Sr}_{0.5}(\text{PO}_4)_2$  and  $\text{Mg}_2\text{Sr}_1(\text{PO}_4)_2$ . All raw powders for material synthesis were mixed five times in a planetary ball mill (PM400 Retsch, Haan, Germany) for 1 h at 200 rpm. Farringtonite ( $\text{Mg}_3(\text{PO}_4)_2$ ) was sintered with a 2:1 M ratio of  $\text{MgHPO}_4 \cdot 3\text{H}_2\text{O}$  (<125  $\mu\text{m}$ , Sigma–Aldrich, Steinheim, Germany) and  $\text{Mg}(\text{OH})_2$  (VWR, Radnor, USA). The composition for  $\text{Mg}_{2.5}\text{Sr}_{0.5}(\text{PO}_4)_2$  was a 4:1:1 M ratio of  $\text{MgHPO}_4 \cdot 3\text{H}_2\text{O}$ ,  $\text{Mg}(\text{OH})_2$  and  $\text{SrCO}_3$  (Sigma–Aldrich, Steinheim, Germany) respectively; similarly a 2:1 M ratio of  $\text{MgHPO}_4 \cdot 3\text{H}_2\text{O}$  and  $\text{SrCO}_3$  yielded the composition  $\text{Mg}_2\text{Sr}_1(\text{PO}_4)_2$ . Powders were sintered in a furnace (Oyten Thermotechnic, Oyten, Germany) at 1050 °C in air for 5 h. The sintered cakes were crushed with pestle and mortar and sieved <355  $\mu\text{m}$ , to obtain the powder mix for three-dimensional printing experiments.

The three dimensional printing of the powder mix was performed on a spectrum Z510 printer (Z-Corporation, Burlington, USA). Each powder was supplemented with 5 wt% (hydroxypro

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