



(51) International Patent Classification:

A61L 27/10 (2006.01) C04B 35/00 (2006.01)
A61L 27/40 (2006.01) C04B 35/624 (2006.01)
A61F 2/28 (2006.01) C01B 33/22 (2006.01)

(21) International Application Number:

PCT/AU2013/000498

(22) International Filing Date:

14 May 2013 (14.05.2013)

(25) Filing Language:

English

(26) Publication Language:

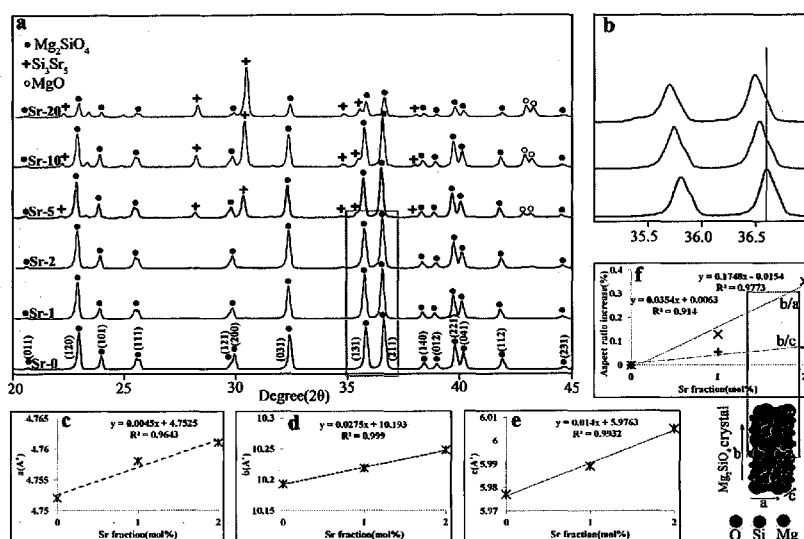
English

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Elizabeth Street, Sydney, New South Wales 2000 (AU).(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP,
KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD,
ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI,
NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU,
RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ,
TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA,
ZM, ZW.(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ,
UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ,
TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK,
EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,
MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM,

[Continued on next page]

(54) Title: A BIOACTIVE MATERIAL AND METHOD OF FORMING SAME

Figure 1

(57) Abstract: A Magnesium Silicate
based biocompatible ceramic material
that degrades to simultaneously release
bioactive ions, and, a method of produ-
cing same.

WO 2014/183148 A1



TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, **Published:**
ML, MR, NE, SN, TD, TG).

— *with international search report (Art. 21(3))*

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A BIOACTIVE MATERIAL AND METHOD OF FORMING SAME

Field of the Invention

[001] The present invention relates to a material, and in particular a Magnesium Silicate based biocompatible ceramic material that degrades to simultaneously release bioactive ions, and, a method of producing same.

Background

[002] Bone mineral is frequently described as Hydroxyapatite. However, it is actually Hydroxyapatite highly modified by the substitution of Calcium with cations such as Strontium and Magnesium, and the substitution of phosphate and hydroxyl groups with anions such as Silicate and Carbonate.

[003] The development of biocompatible materials that contain and can release Strontium, Silicon, and Magnesium to bone sites has received intense interest in recent years for a number of reasons.

[004] Strontium can be readily incorporated into bone mineral, and typically, 98% of the total body Strontium content can be found in the skeleton and is highly bioactive. Strontium Ranelate is presently being used as a new treatment for osteoporosis that has both antiresorptive and anabolic effects. Strontium is incorporated into bone by two mechanisms: (a) surface exchange involving the incorporation of Strontium into the crystal lattice of the bone mineral and (b) ionic substitution whereby Strontium is taken up by ionic exchange with bone Calcium. *In vitro* studies have revealed that the presence of Strontium ions result in increased collagen and non-collagen protein synthesis during early osteoblast differentiation and in inhibition of osteoclast differentiation and function. Further to this, Strontium has also been demonstrated to induce an antibacterial effect. Among the trace metals present in human bone, Strontium is the only one that was correlated with bone compressive strength.

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[005] Silicon is an essential element for metabolic processes associated with the formation and calcification of bone tissue and is present in bone as silicate. High Silicon content has been detected in early stages of bone matrix calcification. Dietary Si intake correlates with bone mineral density (BMD) in men and premenopausal women.

[006] Magnesium is the fourth most abundant cation in the human body, reported to make up 0.44 wt% of enamel, 1.23 wt% of dentin and 0.72% of bone and a link is suggested between Mg deficiency and osteoporosis. Mg has been shown to be involved in bone remodelling and metabolism, the promotion of angiogenesis, and in the growth and mineralization of bone tissue.

[007] Research efforts have concentrated on incorporating these therapeutic ions (Sr, Si and Mg) into different biomaterials such as calcium phosphates, bioactive glasses and calcium silicates. Despite the numerous reports on incorporating bioactive ions into ceramic structures and their release to the aqueous environment, there are still some concerns regarding the efficacy of these devices. Such concerns include: 1) controllability of the ion release rate; 2) ability to simultaneously release multiple ions, and 3) ability to reach therapeutic concentrations in adjacent tissues.

[008] The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in Australia, or elsewhere.

Summary of Invention

[009] In one broad form the present invention provides, a material including a magnesium silicate and strontium.

[010] In one form, the Magnesium Silicate is Mg_2SiO_4 .

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[011] In one form, the material includes an X_{Sr} value of greater than or equal to about 0.02 wherein:

$$X_{Sr} = Sr / (Sr + Mg);$$

Sr = Number of moles Strontium; and

Mg = Number of moles Magnesium.

[012] In another broad from the present invention provides a material including Magnesium, Strontium and Silicon wherein the material has an X_{Sr} value of greater than or equal to about 0.02 wherein:

$$X_{Sr} = Sr / (Sr + Mg);$$

Sr = Number of moles Strontium; and

Mg = Number of moles Magnesium.

[013] In one form, the material has an X_{Sr} value of less than about 1, wherein:

$$X_{Sr} = Sr / (Sr + Mg);$$

Sr = Number of moles Strontium; and

Mg = Number of moles Magnesium.

[014] A material as claimed in any one of the preceding claims, wherein the material has an X_{Sr} value of less than or equal to about 0.2, wherein:

$$X_{Sr} = Sr / (Sr + Mg);$$

Sr = Number of moles Strontium; and

Mg = Number of moles Magnesium.

[015] In one form, the material has an X_{Sr} value of greater than or equal to about 0.05 and less than or equal to about 0.15, wherein,

$$X_{Sr} = Sr / (Sr + Mg);$$

Sr = Number of moles Strontium; and

Mg = Number of moles Magnesium.

[016] In another form, the material includes:

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a first phase including Mg_2SiO_4 ;
a second phase including Si_3Sr_5 ; and
a third phase including MgO .

[017] In a further form, the material is a ceramic material.

[018] In another form, the material is a biocompatible material.

[019] In one form, the material is for promoting bone regeneration.

[020] In another form, the dissolution rate of the material is related to the strontium content of the material.

[021] In one form, the material degrades to simultaneously release Strontium, Silicon and Magnesium ions.

[022] In another form, the material is thermally stable up to about at least 1500°C .

[023] In one form, the material is produced using a sol-gel process.

[024] In another form, the material is produced by doping the magnesium silicate with strontium.

[025] In one form, the material is tri-phasic.

[026] In a further form, the material includes 11 - 70wt% Mg_2SiO_4 .

[027] In another form, the material includes 25 - 69wt% SiSr_5 .

[028] In another form, the material includes 5 - 20wt% MgO .

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[029] In a further broad form the present invention provides a bone scaffold including a material as described in any one of the above forms.

[030] In a further broad form the present invention provides a bone tissue regeneration agent including a material as described in any one of the above forms.

[031] In a further broad form, the present invention provides a method for producing a material as described in any one of the above forms, wherein the method includes a sol-gel process.

[032] In a further broad form the present invention provides a method of promoting bone regeneration, the method including applying a material as described in any one of the above forms to damaged or unhealthy bone.

[033] In a further broad form, the present invention provides a method of adjusting the dissolution rate of a Magnesium Silicate, the method including adding Strontium to the Magnesium Silicate.

[034] In a further form, the Magnesium Silicate is Mg_2SiO_4 .

[035] In a further broad form the present invention provides an injectable composition, the composition including the material as described in any one of the above forms.

[036] In one form the injectable composition includes a polymer and/or a ceramic material.

[037] In a further broad form the present invention provides a composite material including a polymer and a material as described in any one of the above forms.

Definitions

[038] The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an antimicrobial agent" means one antimicrobial agent or more than one antimicrobial agent.

[039] Documents or patent applications referred to within this specification are included herein, in their entirety, by way of reference.

[040] In the context of this specification, the term "about" is understood to refer to a range of numbers that a person of skill in the art would consider equivalent to the recited value in the context of achieving the same function or result.

[041] The term "ceramic" as it is used herein is intended to encompass materials having a crystalline or at least partially crystalline structure formed essentially from inorganic and non-metallic compounds. They are generally formed from a molten mass that solidifies on cooling or are formed and either simultaneously or subsequently matured (sintered) by heating. Clay, glass, cement and porcelain products all fall within the category of ceramics and classes of ceramics include, for example, oxides, silicates, silicides, nitrides, carbides and phosphates.

Brief Description of the Drawings

[042] The present invention will become better understood from the following detailed description of various non-limiting embodiments thereof, described in connection with the accompanying figures, wherein:

[043] Figure 1 (a) and (b) show X-ray diffraction patterns for $X_{Sr} = 0$, $X_{Sr} = 0.01$, $X_{Sr} = 0.02$, $X_{Sr} = 0.05$, $X_{Sr} = 0.1$ and $X_{Sr} = 0.2$ scaffolds;

[044] Figure 1 (c) shows the effect of Strontium on lattice parameter a;

[045] Figure 1 (d) shows the effect of Strontium on lattice parameter b;

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[046] Figure 1 (e) shows the effect of Strontium on lattice parameter V;

[047] Figure 1 (f) shows the aspect ratios of the of Mg_2SiO_4 crystal;

[048] Figures 2 (a) and (d) show pore morphology and strut microstructure of $X_{\text{Sr}} = 0$ scaffolds;

[049] Figures 2 (b) and (e) show pore morphology and strut microstructure of $X_{\text{Sr}} = 0.02$ scaffolds;

[050] Figures 2 (c) and (f) show pore morphology and strut microstructure $X_{\text{Sr}} = 0.10$ scaffolds;

[051] Figures 2 (g), (h) and (i) show SEM images over 60–40 μm area on $X_{\text{Sr}} = 0.10$ scaffolds taken with secondary electron detector and EDX spectral maps as follows: (g) Si (green); (h) Mg (pink); and (i) Sr (red);

[052] Figure 3(a) shows concentrations of Sr released from the $X_{\text{Sr}} = 0$, $X_{\text{Sr}} = 0.01$, $X_{\text{Sr}} = 0.02$, $X_{\text{Sr}} = 0.05$, $X_{\text{Sr}} = 0.1$ and $X_{\text{Sr}} = 0.2$ scaffolds into culture medium after 1, 3, 7, 14, 21 and 28 days of soaking;

[053] Figure 3(b) shows concentrations of Mg released from the $X_{\text{Sr}} = 0$, $X_{\text{Sr}} = 0.01$, $X_{\text{Sr}} = 0.02$, $X_{\text{Sr}} = 0.05$, $X_{\text{Sr}} = 0.1$ and $X_{\text{Sr}} = 0.2$ scaffolds into culture medium after 1, 3, 7, 14, 21 and 28 days of soaking;

[054] Figure 3(c) shows concentrations of Si released from the $X_{\text{Sr}} = 0$, $X_{\text{Sr}} = 0.01$, $X_{\text{Sr}} = 0.02$, $X_{\text{Sr}} = 0.05$, $X_{\text{Sr}} = 0.1$ and $X_{\text{Sr}} = 0.2$ scaffolds into culture medium after 1, 3, 7, 14, 21 and 28 days of soaking;

[055] Figure 3(d) shows pH changes of SBF at different time periods;

[056] Figure 3(e) shows weight loss of the scaffolds after soaking the scaffolds at different time periods;

[057] Figure 4 (a) shows the microstructure of $X_{Sr} = 0$ scaffold after 28 days soaking in SBF;

[058] Figures 4 (b), (c) and (d) the microstructure of $X_{Sr} = 0.10$ scaffold after 28 days soaking in SBF;

[059] Figure 5 (a) shows compressive strength of $X_{Sr} = 0$, $X_{Sr} = 0.01$, $X_{Sr} = 0.02$, $X_{Sr} = 0.05$, $X_{Sr} = 0.1$ and $X_{Sr} = 0.2$ scaffolds in different porosities (85%, 74% and 66%);

[060] Figure 5 (b) shows compressive strength of the scaffolds with porosity of 85% after soaking in SBF for different time periods;

[061] Figures 6 (a), (b) and (c) show morphology of cultured HOB on scaffolds wherein (a) $X_{Sr} = 0$, (B) $X_{Sr} = 0.02$ and (c) $X_{Sr} = 0.10$ after 24h showing close adhesion and spreading of the HOB across the ceramic surface (insets: formation of granules on the cell surfaces of Sr-10 scaffolds).

[062] Figure 6 (d) shows proliferation of HOB on HA/TCP, $X_{Sr} = 0$, $X_{Sr} = 0.01$, $X_{Sr} = 0.02$, $X_{Sr} = 0.05$, and $X_{Sr} = 0.1$ scaffolds after 3 and 7 days culture (* $p < 0.05$).

[063] Figures 7 (a) to (d) show HOB osteogenic gene expression profiles cultured on HA/TCP, $X_{Sr} = 0$, $X_{Sr} = 0.02$ and $X_{Sr} = 0.10$ scaffolds, wherein (a) osteopontin (b) osteocalcin (c) Runx2 (d) BSP

[064] Figure 8 shows Table 1: Primers used for qRT-PCR osteogenesis-related genes; and

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[065] Figure 9 shows Table 2: Concentrations of Sr, Mg and Si released from the $X_{Sr} = 0$, $X_{Sr} = 0.01$, $X_{Sr} = 0.02$, $X_{Sr} = 0.05$, $X_{Sr} = 0.1$ and $X_{Sr} = 0.2$ scaffolds into (a) PBS and (b) SBF after 1, 3, 7, 14, 21 and 28 days of soaking.

Detailed Description

[066] The inventors have developed a new material that can be engineered to have a desired degradation rate and simultaneously release bioactive ions (such as, for example, Sr, Si and Mg).

[067] Embodiments of the new material include a Magnesium Silicate and Strontium. The inventors have found that adding Strontium to a Magnesium Silicate material has an effect on the degradation (and/or dissolution) rate of the Silicate material. Furthermore, as the material degrades, bioactive ions of Strontium, Magnesium and Silicon release into the surrounding environment. As previously described, Magnesium, Silicon and Strontium are particularly useful as agents for the promotion of bone growth and regeneration. Accordingly, the presently described invention has numerous potential medical applications, particularly in orthopaedics.

[068] In one particular embodiment the Magnesium Silicate is Mg_2SiO_4 (forsterite). Other particular examples include $MgSiO_3$ (Enstatite) and $Mg_3Si_4O_{10}(OH)_2$ (talc).

[069] Mg_2SiO_4 is a ceramic with a simple structure that only contains Magnesium cations and Silicate groups. While pure Mg_2SiO_4 has been shown to have a good biocompatibility, its degradation rate is extremely low which, for example, when used for promoting bone tissue regeneration, compromises its ability to release bioactive ions (Si or Mg) into the surrounding biological environment or bone tissue regeneration site.

[070] The inventors have shown that doping Mg_2SiO_4 with Strontium alters the material properties including the crystallinity, solubility and biological performance. In particular, it was found that doping with a threshold amount of Strontium results in the formation of a three phase ceramic material which includes a Mg_2SiO_4 phase, a Si_3Sr_5 phase and a MgO

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phase. This triphasic ceramic promotes simultaneous release of Sr, Mg and Si ions into the surrounding microenvironment, and allows ion release rates that can be tailored based on the specific need.

[071] The Inventors have found that Mg, Sr and Si ions are more readily released by the MgO and Si_3Sr_5 phases and therefore, by adjusting the relative fractions of these phases, the pH of the microenvironment, and the release profiles of the Mg, Sr and Si can be controlled. It therefore follows that by changing the amount of Strontium included in the material, the relative proportions of the three phases can be controlled, and consequently, the degradation rate controlled.

[072] Typically, it has been found that by having an X_{Sr} value (where $X_{\text{Sr}} = \text{Sr}(\text{moles}) / [\text{Sr}(\text{moles}) + \text{Mg}(\text{moles})]$) of greater than about 0.02, results in the formation of the MgO and Si_3Sr_5 phases. Furthermore, further increasing the Strontium content increases the proportions of the Si_3Sr_5 and MgO phases. For example:

- An X_{Sr} value of 0.05 results in a material of 70wt% Mg_2SiO_4 , 25wt% Si_3Sr_5 , and 5wt% MgO;
- An X_{Sr} value of 0.1 results in a material of 50wt% Mg_2SiO_4 , 35wt% Si_3Sr_5 , 15wt% MgO; and
- An X_{Sr} value of 0.2 results in a material of 11wt% Mg_2SiO_4 , 69wt% Si_3Sr_5 , 20wt% MgO.

[073] As shown in Figure 3, formation of Si_3Sr_5 (25-69 wt %) and a small amount of MgO (5-20wt %), such as in those scaffold materials having an X_{Sr} value of 0.05, 0.1 or 0.2, is associated with significantly increased rates of degradation. Mg, Strontium and Si ions were actively released from these scaffolds due to the dissolution of MgO and Si_3Sr_5 .

[074] The material is typically produced using a Sol-Gel process wherein a gel is prepared using appropriate Magnesium, Strontium and Silicon precursors. The prepared gel is then dried and calcined and, if the X_{Sr} value is greater than about 0.02, the three phase structure is formed.

[075] The Sol-Gel process allows synthesis of ceramic materials of high purity and homogeneity, and typically includes a process wherein a solid (typically a gel) is formed from a liquid solution of precursors (for example, organometallic precursors). It will be appreciated that the term Sol-Gel process refers to a methodology known and understood by person skilled in the art.

[076] In one example, to prepare the presently described material, TEOS (Tetraethyl orthosilicate, $\text{Si}(\text{OC}_2\text{H}_5)_4$) is used as a precursor for the silicate network. TEOS is mixed with ethanol to hydrolyze the TEOS (opening its SiO arms and making the SiO available for bonding). Magnesium nitrate hexahydrate ($\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$) is added to the SiO-contained solution to provide magnesium to the SiO network. The material is then doped with Strontium (Sr) by adding strontium nitrate into the solution. By controlling the amount of Strontium added, materials of different properties can be formed. The solution may then be stirred to provide more homogeneity, and is typically aged until it turns into an opaque gel. The gel is then dried and calcined to form a powder of the material. As previously noted, if the X_{Sr} value is greater than about 0.02, a three phase structure is formed.

[077] Although the degradation rate of the material is significantly higher when a three phase structure is formed, it is not entirely necessary to increase the degradation rate. The materials having an X_{Sr} value of less than about 0.02, still show a slight increase in their degradation rate (marginal increase in ion release pattern and weight loss) probably due to the disruption of the uniformity of the crystal structure. Mg_2SiO_4 has a dense structure consisting of the anion SiO_4^{4-} and the cation Mg^{2+} in an orthorhombic crystal structure. Mg^{2+} ions are located in two different spaces of the lattice where one space is larger and uniform (in b direction) compared to that in the c and a direction, and therefore can be a potential site for substitution by larger ions. However the strong repulsion forces between the oxygen atoms results in the crystal structure being adapted in a manner that minimizes these forces.

[078] Although having a X_{Sr} content of less than 0.02 did not result in the formation of any new phases, the crystal aspect ratio and volume parameters increased linearly with increasing amounts of Strontium and also grain morphology changed significantly compared to pure

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Mg_2SiO_4 . This is due to the substitution of small atoms by larger atoms with ionic radius of Strontium being approximately 1.5 times higher than that of Mg. The increasing aspect ratio of the crystal (b/a and b/c) can be attributed to a preferred substitution of Mg ions located in b direction.

[079] It is believed that the addition of larger amounts of Strontium, for example in the range where $X_{\text{Sr}} > 0.02$, results in further instability of the Mg_2SiO_4 structure, resulting in the formation of Si_3Sr_5 and MgO phases upon sintering.

[080] It will be appreciated that the X_{Sr} may be selected in accordance with a desired application/functionality/rate of degradation of the material. For example the material may have $a < X_{\text{Sr}} < b$ where $a = 0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.2, 0.21, 0.22, 0.23, 0.24, 0.25, 0.26, 0.27, 0.28, 0.29, 0.3, 0.31, 0.32, 0.33, 0.34, 0.35, 0.36, 0.37, 0.38, 0.39, 0.4, 0.41, 0.42, 0.43, 0.44, 0.45, 0.46, 0.47, 0.48, 0.49, 0.5, 0.51, 0.52, 0.53, 0.54, 0.55, 0.56, 0.57, 0.58, 0.59, 0.6, 0.61, 0.62, 0.63, 0.64, 0.65, 0.66, 0.67, 0.68, 0.69, 0.7, 0.71, 0.72, 0.73, 0.74, 0.75, 0.76, 0.77, 0.78, 0.79, 0.8, 0.81, 0.82, 0.83, 0.84, 0.85, 0.86, 0.87, 0.88, 0.89, 0.9, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, \text{ or } 0.99$ and $b = 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.2, 0.21, 0.22, 0.23, 0.24, 0.25, 0.26, 0.27, 0.28, 0.29, 0.3, 0.31, 0.32, 0.33, 0.34, 0.35, 0.36, 0.37, 0.38, 0.39, 0.4, 0.41, 0.42, 0.43, 0.44, 0.45, 0.46, 0.47, 0.48, 0.49, 0.5, 0.51, 0.52, 0.53, 0.54, 0.55, 0.56, 0.57, 0.58, 0.59, 0.6, 0.61, 0.62, 0.63, 0.64, 0.65, 0.66, 0.67, 0.68, 0.69, 0.7, 0.71, 0.72, 0.73, 0.74, 0.75, 0.76, 0.77, 0.78, 0.79, 0.8, 0.81, 0.82, 0.83, 0.84, 0.85, 0.86, 0.87, 0.88, 0.89, 0.9, 0.91, 0.92, 0.93, 0.94, 0.95, 0.96, 0.97, 0.98, 0.99, \text{ or } 1$.

[081] Preferred forms of the material have $0.02 < X_{\text{Sr}} < 0.2$. More preferred forms have $0.05 < X_{\text{Sr}} < 0.15$.

[082] Applicants have successfully developed a material, and in particular a triphasic ceramic (containing Mg_2SiO_4 , Si_3Sr_5 and MgO phases), which can simultaneously release

Strontium, Mg and Si ions into the microenvironment. Furthermore by adjusting the proportions of these phases, the release rates of the ions can be controlled.

[083] The release of these ions has the potential to enhance osteogenesis and in some cases angiogenesis within scaffold materials enhancing their potential as therapeutic agents. It is well documented that these ions are involved in bone metabolism and play physiological roles in angiogenesis and in the growth and mineralization of bone tissue. Previous studies have shown that ionic dissolution products, especially Si, from bioactive glasses possesses the capacity to activate osteoblast related gene expression as well as to stimulate osteoblast proliferation and differentiation. The *in vitro* findings, as described in the Examples, support this view.

[084] Furthermore, the attachment, morphology, proliferation and differentiation of primary human bone cells (HOB) were investigated after cell culturing on the various scaffolds. The inventors results showed significant enhancement in osteogenic gene expression levels (Runx2, osteocalcin, osteopontin and bone sialoprotein) when HOB were cultured on materials in accordance with the invention as opposed to other conventional scaffolds such as HA/TCP.

[085] Accordingly it is readily apparent that the material described herein has potential application in the medical field, and in particular, in orthopaedic applications such as, for example, bone tissue regeneration.

[086] It will also be appreciated that the material as described herein may form part of an injectable composition. For example, a powder form of the material may be mixed with a settable polymeric or ceramic material (or other carrier, slurry, ink, paste or putty etc) such as, for example, PCL (polyaprolactone), PMMA (Poly(methyl methacrylate)), a calcium phosphate based cement and/ or a hydrogel system. The composition (including the material as hereindescribed) could then be administered, using a syringe or gun, directly to the site of bone regeneration or implant fixation. This may provide advantages to the injectable systems, such as, for example, improved bioactivity, mechanical properties and X-ray opacity.

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[087] Furthermore it will be appreciated that the material may have other application associated with pH control. For example there are some types of biocompatible and biresorbable polymeric biomaterials such as Poly-L-lactide acid (PLLA), polyglycolic acid (PGA) and their copolymers (PLGA) which are being used extensively in soft and hard tissue engineering. These polymers are considered as biodegradable polymers, meaning they can retain the tissue supporting property for a specific length time and then gradually start degrading. However, the major drawback of these polymers is the release of acidic degradation products which may be potentially toxic and lead to an inflammatory response. A PLLA film can decrease the pH of a culture medium to the acidic level as low as 4 after 14 days. One potential method to address this issue is to make a composite of these polymers and bioactive ceramic materials according to the invention. Materials according to the invention can produce an alkaline environment and could buffer the acidic degradation of these polymers. By increasing the X_{Sr} towards 0.9, the ceramic has ability to increase pH of aqueous environment to 10-11. Such composite materials can be shaped to form prosthetic implants for orthopaedic applications such as screws, fracture bone plates, etc. They can also be manufactured into a porous scaffold for cartilage or bone regeneration applications.

[088] Specific embodiments and supporting experimentation is described in the following Example.

EXAMPLE 1

Preparation and characterization of ceramic powders and scaffolds

[089] Magnesium nitrate hexahydrate ($Mg(NO_3)_2 \cdot 6H_2O$, Sigma-Aldrich), tetraethyl orthosilicate (TEOS, $Si(OC_2H_5)_4$, Sigma-Aldrich) and strontium nitrate ($Sr(NO_3)_2$, Sigma-Aldrich) were used as starting magnesium, silicon and strontium precursors. TEOS was mixed with ethanol (volume ratio: TEOS/ethanol=1:3) and hydrolyzed for 1h under stirring. The solutions were prepared by dissolving appropriate amounts of $Mg(NO_3)_2 \cdot 6H_2O$ and $Sr(NO_3)_2$ in hydrolyzed TEOS at different $X_{Sr}=Sr/(Sr + Mg)$ molar ratios to prepare powder with at different Sr contents ($X_{Sr}=0, 0.01, 0.02, 0.05, 0.1$ and 0.2) and stirred for 2 h

at room temperature. This stirred solution was aged at 65°C for 12 h to form a gel. The gel was dried at 100°C overnight and calcined at 1400°C for 3h min in air using an electrical furnace. For simplicity the materials prepared are named herein based on the X_{Sr} values, i.e. they are referred to as $X_{Sr} = 0$, $X_{Sr} = 0.01$, $X_{Sr} = 0.02$, $X_{Sr} = 0.05$, $X_{Sr} = 0.1$ and $X_{Sr} = 0.2$ scaffolds. Phase Structure analyses of obtained powders and determination of lattice parameters were carried out by X-ray diffractometer (XRD, Siemens D6000, Germany) using Cu K_{α} radiation with a scanning speed of 1°/min and step size of 0.01° over 2θ range of 10 to 80°. Calcium phosphate deficient apatite powder was prepared by an aqueous precipitation reaction. Briefly, $Ca(NO_3)_2 \cdot 4H_2O$ (0.92 M) and $(NH_4)_2HPO_4$ (0.58 M) solutions were mixed gradually at room temperature and pH 11. The precipitated powder was thermally treated at 900°C for 1h, to produce hydroxyapatite/ β -tricalcium phosphate (HA/TCP) powder composed of approximately 40% HA and 60% β -TCP. The powders were ground by mortar and pestle and ball mill to an average size of 5 μ m. A standard stainless steel sieve (25 μ m) was used select only powders smaller than 25 μ m. Scanning electron microscopy and image analysis methods (Image J, NIH) were used to measure the particle size distribution. Fully reticulated polyurethane foam was used as a sacrificial template for scaffold replication via the polymer sponge method. The ceramic slurry was prepared by adding powders to polyvinyl alcohol (PVA) solution to prepare a 30wt% suspension. Foam templates were cut to appropriate dimensions and treated in NaOH solution for 30 min to improve surface hydrophilicity. After cleaning and drying, foams were immersed in the slurry and compressed slightly to facilitate slurry penetration. Excessive slurry was squeezed out and the foam was subsequently blown with compressed air to ensure uniform ceramic coating on the foam surface. The weight of polyurethane foams increased approximately five times after coating with the slurry. After drying at 37°C for 48 h, coated foams were fired in air in an electric furnace using a 4-stage schedule: (i) heating from 25°C to 600°C at a heating rate of 1°C/min, (ii) further heating from 600°C to 1200°C at 2°C/min for HA/TCP and from 600°C to 1450°C at 2°C/min for Mg_2SiO_4 scaffolds and, (iii) holding the temperature at 1200°C for 2 h for HA/TCP and at 1450°C for 3h for $X_{Sr} = 0$, $X_{Sr} = 0.01$, $X_{Sr} = 0.02$, $X_{Sr} = 0.05$, $X_{Sr} = 0.1$ and $X_{Sr} = 0.2$ scaffolds (iv) cooling to 25°C at a cooling rate of 5 °C/min. The microstructure of the scaffolds was characterized by field emission scanning electron microscopy (FE-SEM) (Zeiss; Carl Zeiss, Germany). Internal structure, porosity and interconnectivity of the

scaffolds were evaluated by micro-computerized tomography (Skyscan 1076, Micro-Computed Tomography).

Degradation study in different solutions

[090] *In vitro* degradation of the scaffolds was investigated by soaking the scaffolds in simulated body fluid (SBF), culture medium (α -Minimal Essential Medium [α -MEM], Gibco Laboratories, USA) and phosphate buffered saline (PBS). Cubic scaffolds (8 mm $\times$ 8 mm $\times$ 8 mm) were immersed in solutions at 37°C for 1, 3, 7, 14, 21 and 28 days at a solid/liquid ratio of 150 mg/L. All scaffolds were held in plastic flasks and sealed. At each time point, the scaffolds were removed, rinsed with Milli-Q water and dried at 100°C for 1 day, after which the final weight of each scaffold was measured. The concentration of the ions in the solutions after soaking was tested using inductive coupled plasma atomic emission spectroscopy (ICP-AES; Perkin Elmer, Optima 3000DV, USA). The weight loss (calculated according to the percentage of initial weight before soaking into SBF) and pH changes results were expressed by as mean $\pm$ SD. Five samples of each type of scaffold were tested per time point for statistical analysis.

Mechanical properties of the scaffolds

[091] Mechanical properties of the scaffolds were determined in dry and wet conditions. For wet conditions, the scaffolds were first soaked in SBF for the same time periods as in the degradation study. The compressive strength was determined by crushing cubic scaffolds (6 mm $\times$ 6 mm $\times$ 12 mm) between two flat plates using a computer-controlled universal testing machine (Instron 8874, UK) with a ramp rate of 0.5 mm min⁻¹. Ten identical specimens from each sample group were used for compressive testing in dry and wet conditions.

Cytocompatibility and osteogenic induction property of the scaffolds:

Scaffold sterilization

[092] Cubic scaffolds 5 $\times$ 5 $\times$ 5 mm were sterilized before cell culture using an autoclave (121°C; 20 mins).

Human osteoblast (HOB) isolation, seeding and culture

[093] Permission to use discarded human tissue was granted by the Human Ethics Committee of the University of Sydney and informed consent was obtained. An established method for culturing osteoblast cells was used. Primary human osteoblasts (HOB) were isolated from normal human trabecular bone as previously described. Briefly, the bone was divided into 1 mm³ pieces, washed several times in PBS, and digested for 90 min at 37°C with 0.02% (w/v) trypsin (Sigma-Aldrich, USA) in PBS. Digested cells were cultured in complete medium containing α -MEM, supplemented with 10 vol% heat-inactivated fetal calf serum (FCS) (Gibco Laboratories, USA), 2 mM l-glutamine (Gibco Laboratories, USA), 25 mM Hepes buffer (Gibco Laboratories, USA), 2 mM sodium pyruvate, 100 U ml⁻¹ penicillin, 100 μ g ml⁻¹ streptomycin (Gibco Laboratories, USA) and 1 mM l-ascorbic acid phosphate magnesium salt (Wako Pure Chemicals, Japan). The cells were cultured at 37°C with 5% CO₂ and complete medium changes were performed every 3 days. All HOB used in the experiments were at passage three. After the cells reached 80–90% confluence they were trypsinized with TrypLE™ Express (Invitrogen) and subsequently suspended in complete medium. For HOB attachment and proliferation studies cells were seeded on the scaffolds at initial cell densities of 1.5×10^5 and 5×10^4 cells per scaffold, respectively, in 90 μ l of cell suspension. For the gene expression study cells were seeded on the scaffolds at an initial cell density of 2×10^5 cells per scaffold in 100 μ l of cell suspension. A suspension of HOB was gently dropped onto the scaffolds ($n = 4$) placed in 24-well plates (untreated, NUNC) and incubated for 90 min at 37°C to allow the cells to attach. Then each scaffold was transferred to a new well and 1.5 ml of culture medium was added for culturing. At the designated time points, HOB on the scaffolds was analyzed for attachment, viability and gene expression. If it was observed that HOB migrated from the scaffolds and were growing on the wells, the scaffolds were transferred to a new well for examinations.

HOB attachment and proliferation

[094] HOB attachment was evaluated after 2 and 24 h culture. At each time point scaffolds were prepared for scanning electron microscopy (SEM) (Carl Zeiss, Germany) examination. Scaffolds with cells were fixed with 4% paraformaldehyde solution, post-fixed with 1% osmium tetroxide in PBS for 1 h, dehydrated in graded ethanol (30%, 50%, 70%, 95% and

100%), dried in hexamethyldisilazane for 3 min and then desiccated overnight. The scaffolds were gold sputtered prior to SEM examination. To evaluate HOB proliferation the CellTiter 96 Aqueous Assay (Promega, USA) was used to determine the number of viable cells on the cultured scaffolds via a colorimetric method. The assay solution is a combination of tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium), MTS) with an electron coupling reagent (phenazine methosulfate, PMS) at a volume ratio of 20:1. The former compound can be bio-reduced by viable cells into formazan, which is soluble in cell culture medium, and the absorbance of formazan at 490 nm is directly proportional to the number of viable cells present. HOB proliferation was evaluated after 1 and 7 days culture. At each time point the culture medium was replaced by 1.5 ml of the MTS working solution, which consisted of the CellTiter 96 Aqueous Assay solution diluted in PBS at a volume ratio of 1:5. After 4 h incubation at 37 °C 100 µl of the working solution was transferred to a 96-well cell culture plate and the absorbance at 490 nm was recorded using a microplate reader (PathTech, Australia) using the software Accent (Australia).

HOB gene expression

[095] Quantitative real time polymerase chain reaction (qRT-PCR) was used to evaluate osteogenic gene expression on the cultured scaffolds. Total RNA was isolated from HOB cultured on each scaffold using Trizol (Sigma-Aldrich, USA) and purified using the RNeasy Mini Kit (Qiagen, USA) according to the manufacturer's instructions. First strand cDNA was synthesized from 0.7 µg total RNA using an Omniscript RT Kit (Qiagen, USA) according to the manufacturer's instructions. The cDNA was analyzed for the expression of osteoblast-specific genes, specifically Runx2, collagen type I, bone sialoprotein and osteocalcin, and their expression was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) to obtain relative gene expression. The primers for the selected genes are listed in Table 1.

Statistics

[096] All data are presented as means ± SD and were derived from at least four independent samples. For statistical analysis Levene's test was performed to determine the homogeneity of variance of the data, and then either Tukey's HSD or Tamhane's post hoc tests were used.

The PASW statistics program was employed for all statistical analyses and differences were considered as significant for $p < 0.05$.

Results

[097] The X-ray diffraction patterns and crystallographic analysis of the prepared groups are shown in Figure 1(a) to (f). Characteristic peaks of pure Mg_2SiO_4 were detected for $X_{\text{Sr}} = 0$, $X_{\text{Sr}} = 0.01$ and $X_{\text{Sr}} = 0.02$ scaffolds with the diffraction peaks for $X_{\text{Sr}} = 0.01$ and $X_{\text{Sr}} = 0.02$ shifting to lower 2θ values (Fig.1b), indicating an increase in d-spacings and hence lattice parameters. The addition of Sr (up to $X_{\text{Sr}} = 0.02$) caused a linear increase of lattice parameters (a, b and c) as well as unit cell volume (V) (Fig.1c-e) (R^2 values > 0.9). More importantly, the aspect ratios of Mg_2SiO_4 unit cell (b/a and b/c) increased linearly ($R^2 > 0.9$) by increasing Sr up to $X_{\text{Sr}} = 0.02$ (Fig. 1f), and the amount of this increase for (b/a) was significantly higher (~5 times) than that for (b/c). In contrast, other phases (MgO and Sr_3Si_5) were detected for $X_{\text{Sr}} = 0.05$, $X_{\text{Sr}} = 0.1$ and $X_{\text{Sr}} = 0.2$ scaffolds as shown in the X-ray patterns (Fig. 1a) with incremental increase in peak intensity of MgO and Sr_3Si_5 from $X_{\text{Sr}} = 0.05$ to $X_{\text{Sr}} = 0.2$, while that for Mg_2SiO_4 decreased. Thus $X_{\text{Sr}} = 0$, $X_{\text{Sr}} = 0.01$ and $X_{\text{Sr}} = 0.02$ had the same phase composition as Mg_2SiO_4 . Increased Sr content resulted in the formation of a new chemical composition where $X_{\text{Sr}} = 0.05$ had a composition of (70wt%) Mg_2SiO_4 , (25wt%) Si_3Sr_5 , (5wt%) MgO ; $X_{\text{Sr}} = 0.1$ had a composition of (50wt%) Mg_2SiO_4 , (35wt%) Si_3Sr_5 , (15wt%) MgO ; $X_{\text{Sr}} = 0.2$ has a composition of (11wt%) Mg_2SiO_4 , (69wt%) Si_3Sr_5 , (20wt%) MgO .

[098] SEM examination revealed the highly porous (porosity: ~85% and pore size: $\approx 400 \mu\text{m}$) structure for all the prepared scaffolds with $\approx 100\%$ pore interconnectivity (Fig. 2a-c). Figure 2d, e and f show typical strut microstructure of $X_{\text{Sr}} = 0$, $X_{\text{Sr}} = 0.02$ and $X_{\text{Sr}} = 0.1$ ($X_{\text{Sr}} = 0.05$ and $X_{\text{Sr}} = 0.2$ had a similar microstructure to $X_{\text{Sr}} = 0.1$) scaffolds, respectively. $X_{\text{Sr}} = 0$ microstructure consisted of the equiaxed grains with average size of $1.5 \mu\text{m}$ (Fig. 2d), however $X_{\text{Sr}} = 0.02$ microstructure consisted of elongated grains with a preferred growth direction (Fig. 2e). Three different crystalline grains could be observed in the $X_{\text{Sr}} = 0.1$ microstructure (Fig. 2d): I-Cuboidal-shaped grains which are scattered in small amounts in the triple junctions or inside the other grains with average size of 350nm; II-large prismatic

shaped grains with average size of $3\mu\text{m}$ and III-equiaxed grains with average size of $700\mu\text{m}$ dispersed between the prismatic shaped grains.

[099] Elemental distribution mapping was carried out to evaluate the qualitative distribution of Si, Mg and Sr elements in the $X_{\text{Sr}} = 0.1$ scaffolds microstructure (Fig. 2g-i). Results demonstrated a striking difference in Sr and Mg distribution, where Mg was localized in types I and II grains but Sr only existed in the type II grains. The map shows the presence of Si elements in the major parts of the microstructure except for type I grains.

[0100] Figure 3 shows the degradation behaviour of prepared scaffolds when incubated with culture medium; PBS and in SBF solutions for different time periods. Fig. 3a-c depicts the concentrations of Sr, Mg and Si released from all the scaffolds incubated in culture medium for 0, 7, 14 and 21 days. Typical two-step release kinetics was obtained for the scaffolds where the release of ions proceeded rapidly in the first 3 days followed by a decreased release rate. An initial fast release can be observed however after that initial burst, a sustained slow and relatively linear release of ions was observed. For $X_{\text{Sr}} = 0.05$, $X_{\text{Sr}} = 0.1$ and $X_{\text{Sr}} = 0.2$ scaffolds, concentrations of Sr, Mg and Si released into the culture medium were significantly higher than that for the rest of the scaffolds at time points tested.

[0101] More importantly, this trend was maintained after the scaffolds were incubated in PBS or SBF solutions with the following order ($X_{\text{Sr}} = 0.2 > X_{\text{Sr}} = 0.1 > X_{\text{Sr}} = 0.05 >> X_{\text{Sr}} = 0.02 > X_{\text{Sr}} = 0.01 > X_{\text{Sr}} = 0$) (Table 3), with $X_{\text{Sr}} = 0$ scaffolds having the least degradability with Si and Mg concentrations in the culture medium reaching 2.6 ± 0.1 and 4.1 ± 0.2 ppm, respectively, compared to 10.2 ± 0.1 and 81 ± 4 respectively for $X_{\text{Sr}} = 0.1$ scaffolds at 28 days. A slight increase in ion release rate was found for $X_{\text{Sr}} = 0.01$ and $X_{\text{Sr}} = 0.02$, compared to $X_{\text{Sr}} = 0$ scaffolds however the values remained markedly lower than that for $X_{\text{Sr}} = 0.05$, $X_{\text{Sr}} = 0.1$ and $X_{\text{Sr}} = 0.2$ scaffolds. Clearly between 1 to 28 days, $X_{\text{Sr}} = 0.01$, $X_{\text{Sr}} = 0.02$, $X_{\text{Sr}} = 0.1$ and $X_{\text{Sr}} = 0.2$ scaffolds released the Sr into the culture medium according to their Sr content ($X_{\text{Sr}} = 0.01$: from 0.5 ± 0.05 to 1.1 ± 0.07 ppm; $X_{\text{Sr}} = 0.02$: from 0.8 ± 0.12 to 2.4 ± 0.22 ppm; $X_{\text{Sr}} = 0.05$: from 2.3 ± 0.16 to 4.9 ± 0.20 ppm; $X_{\text{Sr}} = 0.1$: from 4.5 ± 0.49 to 10 ± 0.60 ppm and $X_{\text{Sr}} = 0.2$: from 6.5 ± 0.57 to 17 ± 0.87 ppm).

[0102] The pH variation patterns of the SBF solution containing $X_{Sr} = 0$, $X_{Sr} = 0.01$, $X_{Sr} = 0.02$, $X_{Sr} = 0.05$, $X_{Sr} = 0.1$ and $X_{Sr} = 0.2$ scaffolds as a function of time are depicted in Fig. 3d. In agreement with the degradation results, the composition of $X_{Sr} = 0.05$, $X_{Sr} = 0.1$ and $X_{Sr} = 0.2$ scaffolds significantly affected the pH values of the SBF solution. pH remained almost unchanged at 7.4 for $X_{Sr} = 0$, $X_{Sr} = 0.01$ and $X_{Sr} = 0.02$ scaffolds during 28 days of soaking. In contrast, the pH of the SBF containing $X_{Sr} = 0.1$ and $X_{Sr} = 0.2$ scaffolds showed different patterns, with a rapid increase from days 1 to 7, followed by a gradual increase during the four weeks incubation period; stabilising to 8 and 8.6 after 28 days for $X_{Sr} = 0.1$ and $X_{Sr} = 0.2$ scaffolds, respectively. Except for $X_{Sr} = 0$ scaffolds, weights of all the scaffolds in SBF decreased significantly with soaking time (Fig. 3e). After 28 days, weight loss for $X_{Sr} = 0$, $X_{Sr} = 0.01$, $X_{Sr} = 0.02$, $X_{Sr} = 0.05$, $X_{Sr} = 0.1$ and $X_{Sr} = 0.2$ scaffolds reached ~1.1%, ~2.2%, 3.2%, 6.3%, 14.2% and 18.8%, respectively.

[0103] Interestingly the order of weight loss seen paralleled that observed for the release of Sr, Si and Mg ions. Figure 4 shows the surface microstructure of $X_{Sr} = 0$ and $X_{Sr} = 0.1$ scaffolds after 28 days of soaking in SBF solution. The surface microstructure of $X_{Sr} = 0.01$ and $X_{Sr} = 0.02$ scaffolds showed similar features to $X_{Sr} = 0$ scaffolds and surface microstructure of $X_{Sr} = 0.05$ and $X_{Sr} = 0.2$ scaffolds showed similar features to $X_{Sr} = 0.1$ scaffolds after soaking in SBF. The $X_{Sr} = 0$, $X_{Sr} = 0.01$ and $X_{Sr} = 0.02$ scaffold surfaces were free of mineral precipitates and remained almost intact during 28 days of soaking at SBF (Fig. 4a). The surfaces of $X_{Sr} = 0.05$ and $X_{Sr} = 0.1$ scaffolds contained submicron holes, grooves and nanoscale precipitates providing evidence of a degradation process (Fig. 4b). The holes were formed due to dissolution of MgO grains in triple junctions and inside the Sr_3Si_5 grains (Fig. 4c). EDS analysis showed the precipitates (Fig. 4d) mainly consisted of Ca, P, Na, Cl and Mg elements.

[0104] The compressive strength of the scaffolds tested at various porosities (85%, 74% and 66%) was measured, and as expected it was found to increase markedly as the porosity decreased. At a porosity of 85%, the compressive strength for $X_{Sr} = 0$, $X_{Sr} = 0.01$, and $X_{Sr} = 0.02$ scaffolds were 1.15 MPa, 0.9MPa and 0.95MPa respectively, and increased to 14.1 MPa

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and 12.1MPa and 21.7MPa when the porosity decreased to 66%. Compressive strength of Sr-5 and $X_{Sr} = 0.1$ scaffolds at 85% porosity was 0.8MPa and 0.85MPa respectively, and increased to 10.2MPa and 11MPa at the 66% porosity. $X_{Sr} = 0.2$ scaffolds had the lowest compressive strength (0.6 to 6.1 MPa) at porosities of 85% and 66%, respectively. However the compressive strength for all scaffolds tested (regardless of their porosities) remained significantly higher than that for HA/TCP scaffolds. $X_{Sr} = 0$, $X_{Sr} = 0.01$ and $X_{Sr} = 0.02$ showed the least decrease in compressive strength (Fig. 5b), compared to the marked decrease (from 0.6MPa to 0.2MPa) seen for $X_{Sr} = 0.2$ scaffolds and the mild decrease (from 0.8MPa to 0.62) for $X_{Sr} = 0.1$ scaffolds after 28 days of soaking in SBF.

[0105] The modulus of elasticity of all the scaffolds showed similar decreasing pattern to their compressive strength (Fig. 5b). HOB attachment and morphology on the $X_{Sr} = 0$, $X_{Sr} = 0.02$ and $X_{Sr} = 0.10$ scaffolds were examined by SEM (Fig. 6a–b). After 2 h and 24h culture, HOB attached to the surface of the various scaffolds with almost similar morphology with a flattened appearance. At 24h, attached HOB cells on scaffolds were spread out and exhibited an elongated morphology with the formation of extended filopodia. Close observation by SEM showed that some granules have formed on the cell surfaces of $X_{Sr} = 0.10$ scaffolds which may be an indication of early biomineralization.

[0106] Figure 6 shows proliferation of HOB on the HA/TCP, $X_{Sr} = 0$, $X_{Sr} = 0.01$, $X_{Sr} = 0.02$, $X_{Sr} = 0.05$ and $X_{Sr} = 0.1$ scaffolds over the 3 and 7 days of culture. For all of the groups, cell activity increased significantly from 3 to 7 day of culture. After 3 days, the $X_{Sr} = 0.01$ and $X_{Sr} = 0.02$ scaffolds exhibited a significant increase in cell proliferation compared to other groups however the greater substitution of Sr seemed to slow down proliferation. A similar trend was also observed after 7 days.

[0107] Fig. 7 (a-d) shows osteogenic gene expression profiles for HOB cultured on HA/TCP, $X_{Sr} = 0$, $X_{Sr} = 0.02$ and $X_{Sr} = 0.10$ scaffolds. qRT-PCR results showed that the HOB seeded for 3 and/or 7 days on $X_{Sr} = 0.10$ scaffolds expressed significantly higher levels of osteopontin and runx2 than those on HA/TCP, $X_{Sr} = 0$ and $X_{Sr} = 0.02$ scaffolds. Moreover, $X_{Sr} = 0.1$ scaffolds expressed significantly higher levels of BSP (bone sialoprotein) and

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osteocalcin than those on HA/TCP, $X_{Sr} = 0$ and $X_{Sr} = 0.02$ scaffolds at day 7. $X_{Sr} = 0.01$ and $X_{Sr} = 0.02$ scaffolds expressed significantly higher levels of osteopontin, osteocalcin, runx2 and BSP than those on HA/TCP at 3 and/or 7 days.

[0108] The Inventors have shown that it is possible to control simultaneous and active release of these three important ions by changing the fractions of Si_3Sr_5 (25 to 69 wt%), MgO (5 to 20wt%) and Mg_2SiO_4 (70 to 11wt%) in the scaffold. $X_{Sr} = 0.05$, $X_{Sr} = 0.1$ and $X_{Sr} = 0.2$ scaffold gave the best dissolution and degradation rates where Strontium, Mg and Si concentrations in culture medium (after 7 days) were in the range of ~5 to 10ppm, ~10 to 85ppm and ~4 to 8ppm, respectively, with similar ion concentrations observed even after these scaffolds were incubated for 28 days in PBS and SBF. Thus it is reasonable to expect that, *in vivo*, concentration gradients may be formed in the tissues around the developed materials to levels sufficient to enhance material bioactivity.

[0109] In this Example, $X_{Sr} = 0.1$ scaffolds markedly increase the pH to values around 8. While increasing pH *in vivo* has the potential to cause toxicity, it is interesting to mention that recently it has been shown in other studies that osteoblast activity was significantly enhanced with a modest increase in pH to 8-8.5, where the positive effect of strontium on osteoblasts was further increased. Thus small increases in local pH around an implant may not be detrimental.

[0110] qRT-PCR results demonstrated that the HOBs seeded on $X_{Sr} = 0.1$ scaffolds expressed significantly higher levels of osteopontin, osteocalcin, runx2 and bone sialoprotein than those on HA/TCP, $X_{Sr} = 0$ and $X_{Sr} = 0.02$ scaffolds after 7 days. These are genetic markers for osteoblast differentiation, with osteocalcin in particular being a marker of late stages of osteoblast maturation. The observation of slowing of proliferation of HOB grown with $X_{Sr} = 0.1$ is also consistent with differentiation as proliferation and differentiation tend to change inversely. Together these results suggest that osteoblasts can detect osteoinductive signals from $X_{Sr} = 0.1$ scaffolds to promote their differentiation into the osteoblast lineage. However, it remains unclear which ionic products (and at what concentration) were responsible for cell activation and the exact mechanism(s) of interaction between the ionic products and cells. If

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dissolution rates of bioactive materials are too rapid, the ionic concentrations of the fluid environment around the cells will be too high, which will be detrimental to cells. If the rates are too slow, the ion concentrations are too low to stimulate cellular activity.

[0111] As shown in this Example the Inventors have developed a triphasic ceramic with the capability for multiple ion release (Sr, Mg and Si) at rates that can be varied, providing a tool for optimisation. This ceramic showed itself to be an exceptional carrier for releasing the bioactive ions but also attained a compressive strength within and above the range of human cancellous bone (0.6–15 MPa) at corresponded porosity. Moreover, they provided significantly increased rates of degradation and bioactivity compared to HA/TCP scaffolds and thus could be of interest for use in a wide range of orthopaedic applications such as bone void fillers, porous scaffolds for bone tissue engineering applications.

[0112] The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as, an acknowledgement or admission or any form of suggestion that the prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

[0113] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

[0114] Finally, it is to be understood that various alterations, modifications and/or additions may be incorporated into the various constructions and arrangements of parts without departing from the spirit or ambit of the invention.

The Claims

1. A material including a Magnesium Silicate and Strontium.
2. A material as claimed in claim 1, wherein the Magnesium Silicate is Mg_2SiO_4 .
3. A material as claimed in claim 1 or claim 2, wherein the material includes an X_{Sr} value of greater than or equal to about 0.02 wherein:
$$X_{\text{Sr}} = \text{Sr} / (\text{Sr} + \text{Mg});$$

Sr = Number of moles Strontium; and
Mg = Number of moles Magnesium.
4. A material including Magnesium, Strontium and Silicon wherein the material has an X_{Sr} value of greater than or equal to about 0.02 wherein:
$$X_{\text{Sr}} = \text{Sr} / (\text{Sr} + \text{Mg});$$

Sr = Number of moles Strontium; and
Mg = Number of moles Magnesium.
5. A material as claimed in any one of the preceding claims, wherein the material has an X_{Sr} value of less than about 1, wherein:
$$X_{\text{Sr}} = \text{Sr} / (\text{Sr} + \text{Mg});$$

Sr = Number of moles Strontium; and
Mg = Number of moles Magnesium.
6. A material as claimed in any one of the preceding claims, wherein the material has an X_{Sr} value of less than or equal to about 0.2, wherein:
$$X_{\text{Sr}} = \text{Sr} / (\text{Sr} + \text{Mg});$$

Sr = Number of moles Strontium; and
Mg = Number of moles Magnesium.

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7. A material as claimed in any one of the preceding claims, wherein the material has an X_{Sr} value of greater than or equal to 0.05 and less than or equal to 0.15, wherein,
$$X_{Sr} = Sr / (Sr + Mg);$$

 Sr = Number of moles Strontium; and
 Mg = Number of moles Magnesium.
8. A material as claimed in any one of the preceding claims including:
 - a first phase including Mg_2SiO_4 ;
 - a second phase including Si_3Sr_5 ; and
 - a third phase including MgO .
9. A material as claimed in any one of the preceding claims, wherein the material is a ceramic material.
10. A material as claimed in any one of the preceding claims, wherein the material is a biocompatible material.
11. A material as claimed in any one of the preceding claims, wherein the material is for promoting bone regeneration.
12. A material as claimed in any one of the preceding claims, wherein the dissolution rate of the material is related to the strontium content of the material.
13. A material as claimed in any one of the preceding claims, wherein the material degrades to simultaneously release Strontium, Silicon and Magnesium ions.
14. A material as claimed in any one of the preceding claims, wherein the material is thermally stable up to about at least $1500^{\circ}C$.
15. A material as claimed in any one of the preceding claims, wherein the material is produced using a sol-gel process.

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16. A material as claimed in any one of the preceding claims, wherein the material is produced by doping the magnesium silicate with strontium.
17. A material as claimed in any one of the preceding claims, wherein the material is tri-phasic.
18. A material as claimed in claim any one of the preceding claims, wherein the material includes 11 - 70wt% Mg_2SiO_4 .
19. A material as claimed in any one of the preceding claims, wherein the material includes 25 - 69wt% SiSr_5 .
20. A material as claimed in any one of the preceding claims, wherein the material includes 5 - 20wt% MgO .
21. A bone scaffold including a material as claimed in any one of the preceding claims.
22. A bone tissue regeneration agent including a material as claimed in any one of claims 1 to 20.
23. A method for producing a material as claimed in any one of claims 1 to 20, wherein the method includes a sol-gel process.
24. A method of promoting bone regeneration, the method including applying a material as claimed in any one of claims 1 to 20 to damaged or unhealthy bone.
25. A method of adjusting the dissolution rate of a Magnesium Silicate, the method including adding Strontium to the Magnesium Silicate.
26. A method as claimed in claim 25, wherein the Magnesium Silicate is Mg_2SiO_4 .

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27. An injectable composition, the composition including the material as claimed in any one of claims 1 to 20.
28. An injectable composition as claimed in claim 20, wherein the composition includes a polymer and/or a ceramic material.
29. A composite material including a polymer and a material as claimed in any one of claims 1 to 28.

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Figure 1

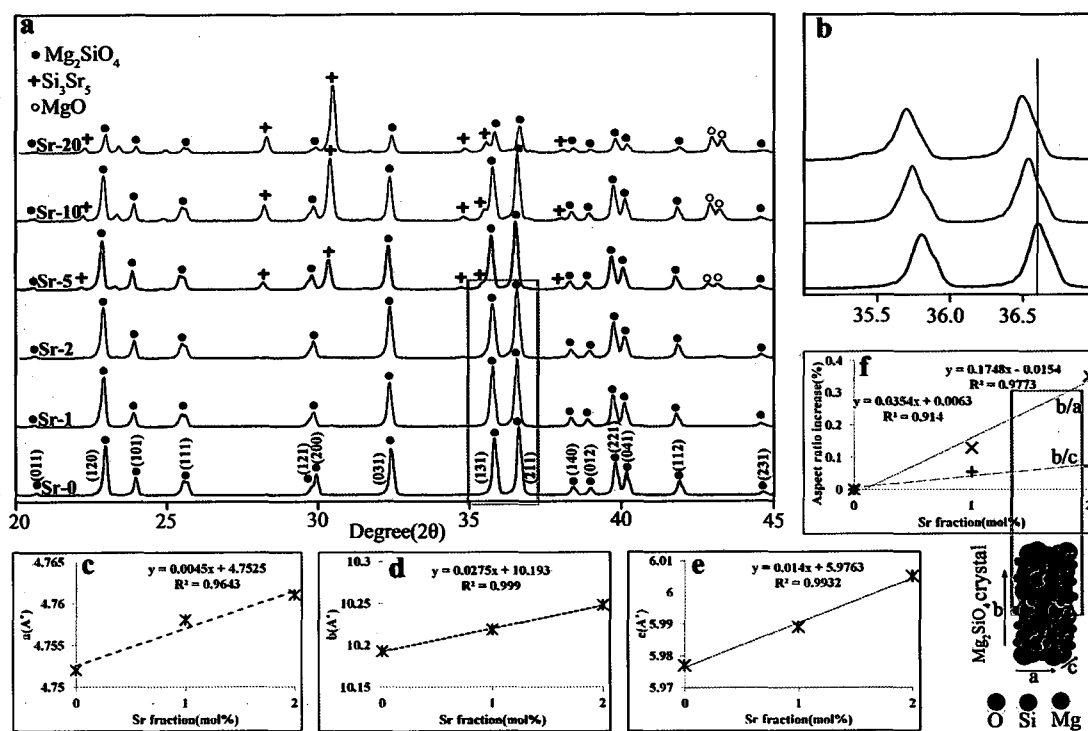


Figure 2

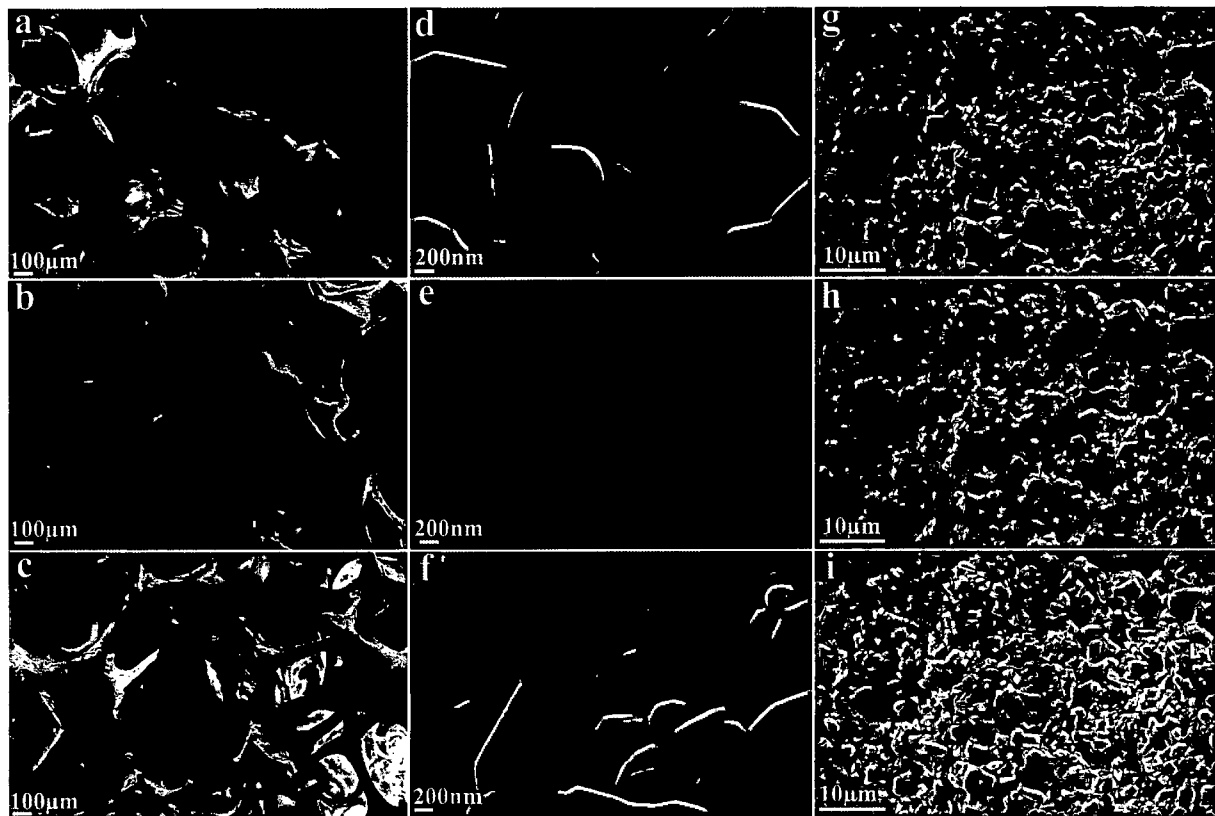


Figure 3

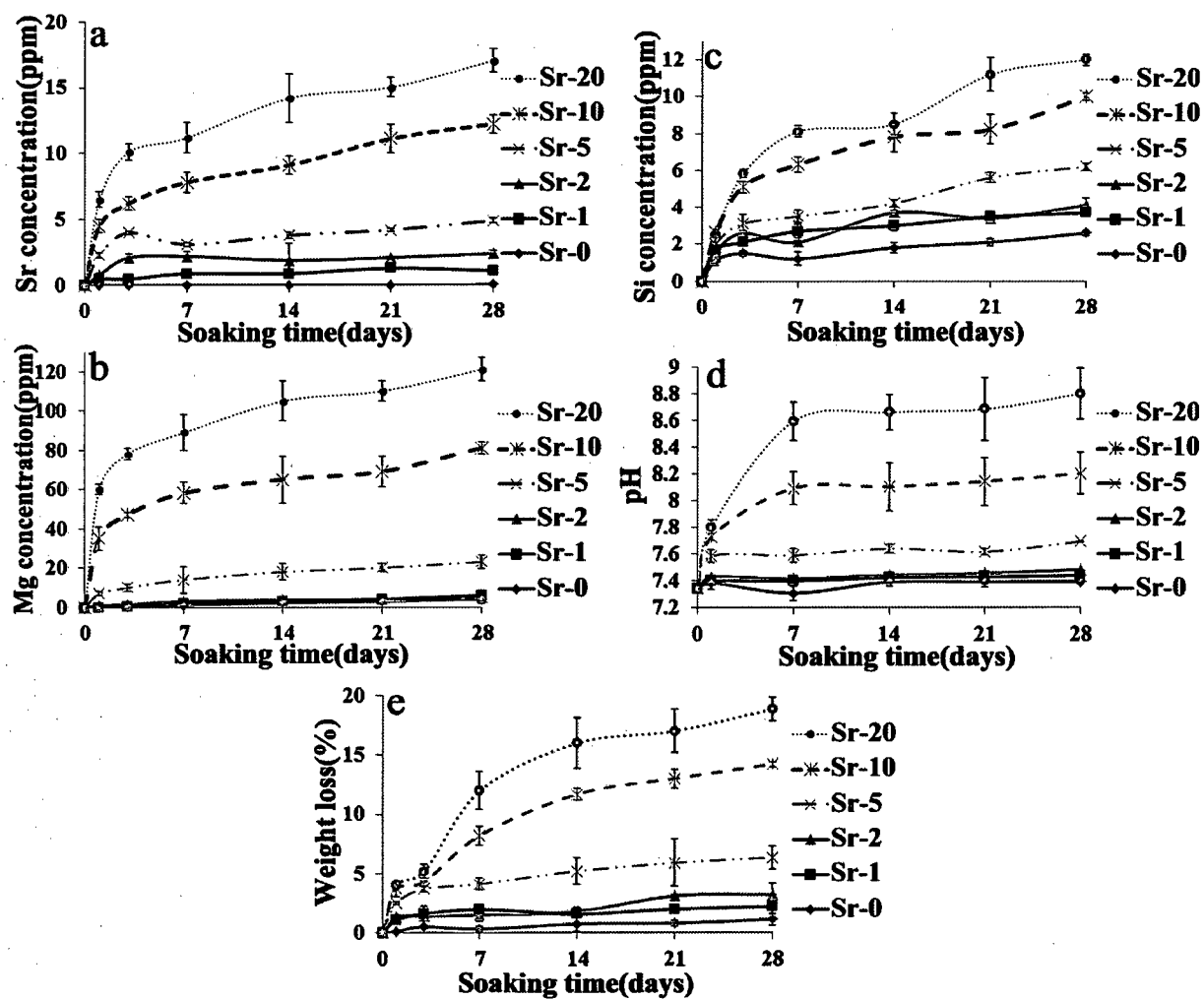


Figure 4

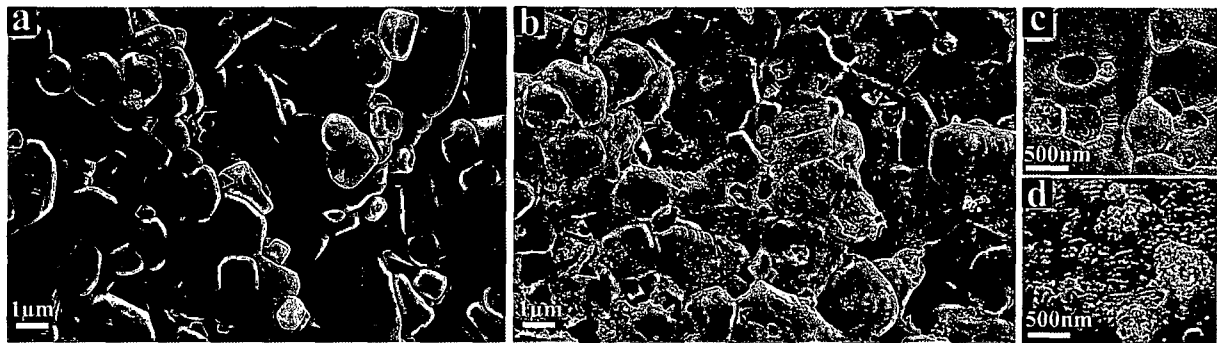


Figure 5

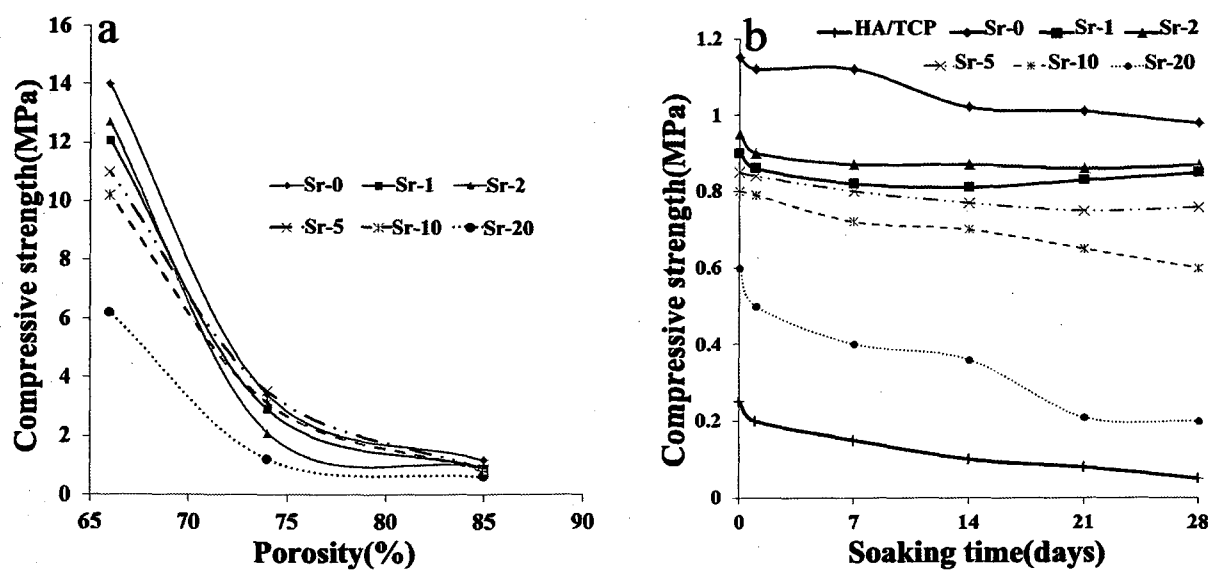


Figure 6

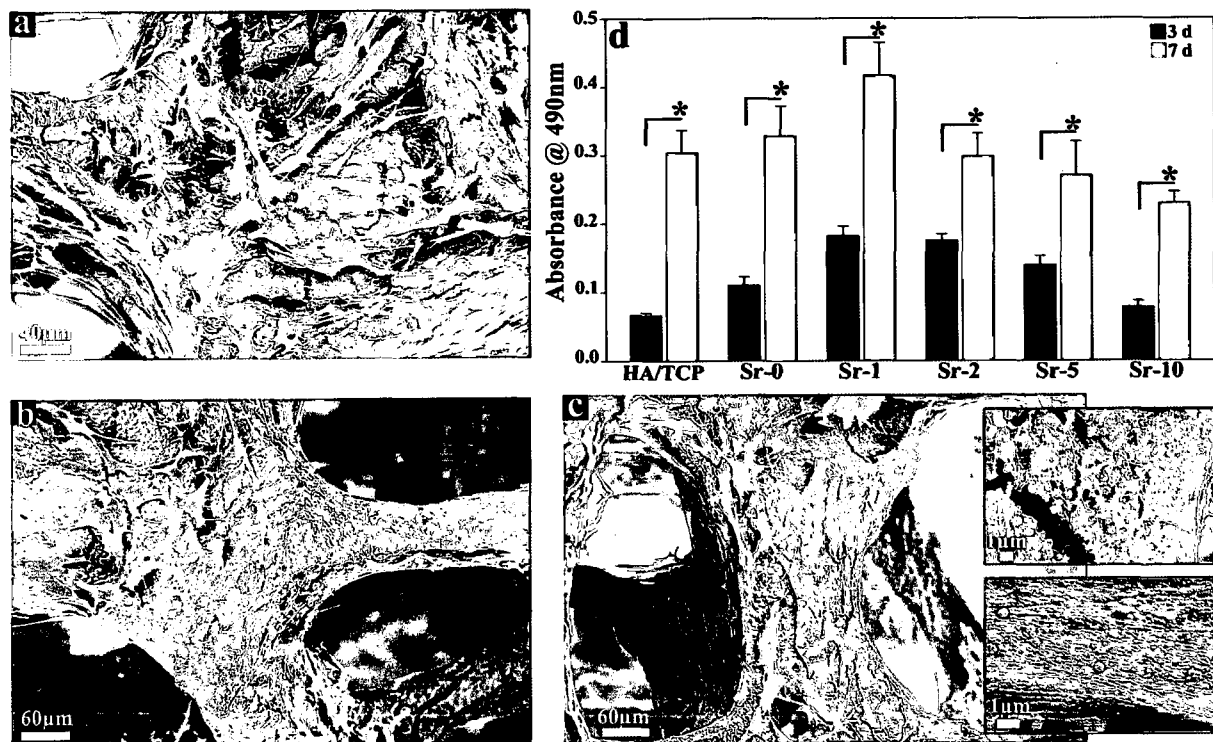


Figure 7

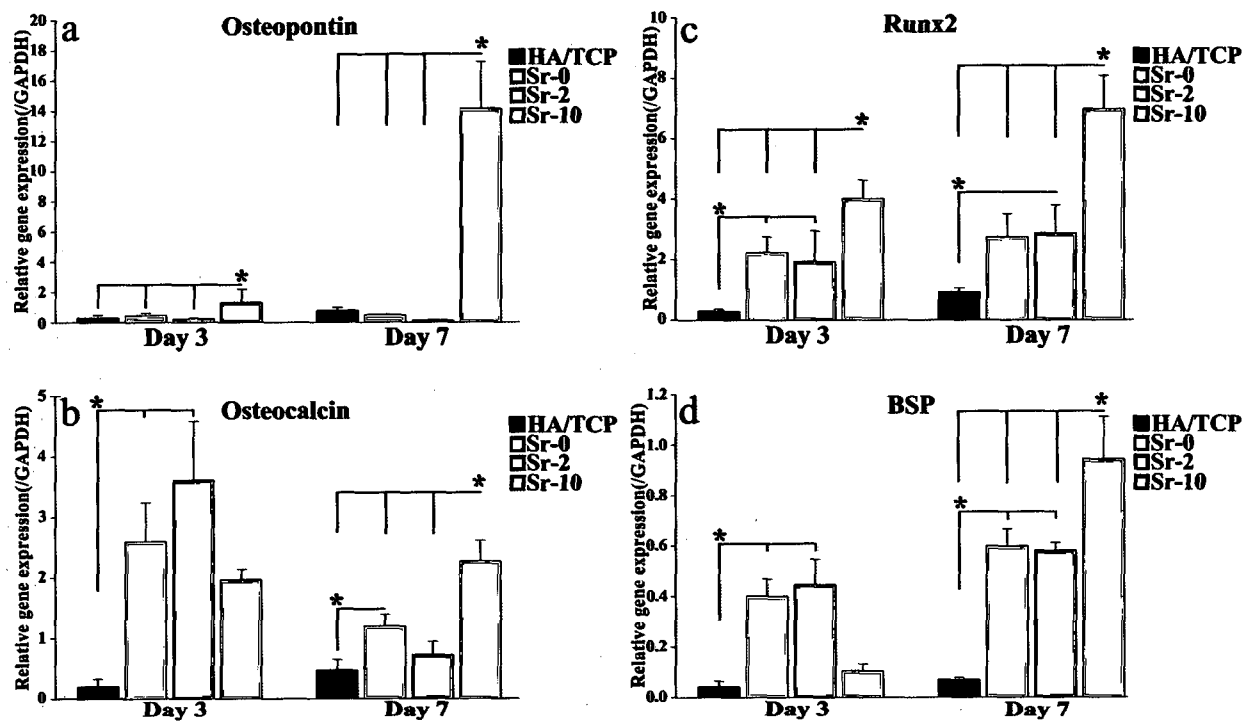


Figure 8 / Table 1

Gene	Sequence (5'-3')		Melting
			Temperature(°C)
GAPDH	F	ACCCAGAAGACTGTGGATGG	60
	R	CAGTGAGCTTCCCGTTCAG	
Runx-2	F	ATGCTTCATTGCGCTCAC	60
	R	ACTGCTTGCAGCCTTAAAT	
Osteopontin	F	TTCCAAGTAAGTCCAACGAAG	60
	R	GTGACCAGTTCATCAGATTTCAT	
Osteocalcin	F	ATGAGAGCCCTCACACTCCTCG	60
	R	GTCAGCCAACCTCGTCACAGTCC	
Bone sialoprotein	F	ATGGCCTGTGCTTTCTCAATG	60
	R	GGATAAAAGTAGGCATGCTTG	

Figure 9 / Table 2

a		1	3	7	14	21	28
Sr-0	Sr	0.0	0.0	0.0	0.00	0.0	0.0
	Mg	0.0	0.0	0.0	0.1	0.2	0.3
	Si	0.1	0.2	0.2	0.3	0.3	0.3
Sr-1	Sr	0.5	0.6	0.9	2.0	2.7	3.8
	Mg	0.0	0.7	0.9	1.0	0.9	1.1
	Si	0.5	0.5	0.6	0.9	0.9	1.0
Sr-2	Sr	0.6	1.0	1.2	1.8	3.8	4.0
	Mg	0.0	2.0	3.0	3.1	3.9	4.2
	Si	0.2	0.3	0.5	0.5	0.7	0.9
Sr-5	Sr	3.4	3.3	3.1	3.6	4.2	5.8
	Mg	26.1	28.4	30.2	34.7	40.1	42.5
	Si	1.0	1.0	1.0	1.3	1.7	1.9
Sr-10	Sr	4.7	7	8.3	8.9	11.1	12.2
	Mg	56.2	100.1	180.4	213.7	230.9	240.2
	Si	1.1	1.0	0.85	1.0	1.5	1.6
Sr-20	Sr	6.8	11	10.1	14.2	17	18.2
	Mg	64.1	210.3	300.4	303.6	320.1	340.8
	Si	1.3	1.4	1.5	1.6	1.8	2.0

b		1	3	7	14	21	28
Sr-0	Sr	0.0	0.0	0.0	0.0	0.0	0.0
	Mg	0.0	0.4	1.1	2.0	2.5	3.3
	Si	2.3	2.5	2.9	2.2	3.0	3.1
Sr-1	Sr	0.5	0.5	0.9	2.0	2.7	3.8
	Mg	0.0	0.8	2.8	3.1	3.9	4.0
	Si	2.7	2.6	3.7	3.7	3.8	4.2
Sr-2	Sr	0.9	2.0	3.1	4.3	4.8	7.0
	Mg	1.2	2.3	3.1	4.2	4.8	5.1
	Si	2.9	2.5	3.9	4.7	4.6	4.8
Sr-5	Sr	4.1	5.2	5.8	8.2	12.3	13.9
	Mg	15.2	18.3	25.6	29.1	31.5	34.2
	Si	4.3	5.1	6.8	7.3	7.8	8.1
Sr-10	Sr	10.4	13.5	14.2	14.2	18.2	20.1
	Mg	46.1	73.4	78.1	93.4	140.1	160.2
	Si	3.4	6.1	7.2	7.8	8.2	8.9
Sr-20	Sr	15.1	16.3	18.6	26.1	25.1	29.1
	Mg	58.2	132.5	189.3	190.1	210.2	220.7
	Si	3.8	5.1	7.6	8.2	8.8	9.2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2013/000498

A. CLASSIFICATION OF SUBJECT MATTER

A61L 27/10 (2006.01) A61L 27/40 (2006.01) A61F 2/28 (2006.01) C04B 35/00 (2006.01) C04B 35/624 (2006.01) C01B 33/22 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Data Base: EPODOC, WPI, CAPLUS, MEDLINE, GOOGLE. Keywords: Magnesium, silicate, strontium, forsterite, talc, enstetite, ceramic, bioglass, bone, scaffold, biocompatible, bioresorbable, bioactive, dop, Si₃Sr₅, MgO, magnesia, sol and gel, implant, graft, resorb, biodegradable and similar

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

☒ Further documents are listed in the continuation of Box C
 ☒ See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
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Date of the actual completion of the international search
8 July 2013Date of mailing of the international search report
08 July 2013

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INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2013/000498
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Information on patent family members		PCT/AU2013/000498	
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