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DIRECCIÓN DE NUEVAS CREACIONES

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Abogado
LUZ CLEMENCIA DE PÁEZ

ASUNTO	Radicación	09-119370
	Trámite	011
	Evento	378
	Actuación	519
	Oficio	
	Folios	012

0.142.00

Patente de Invención	☒	Patente de Modelo de Utilidad	☐
Vía Nacional	☐		
Vía PCT (Fase Nacional)	☐	Cap. I	☐
		Cap. II	☐

No. Solicitud Internacional PCT/US2008/060720
 Fecha de Presentación Internacional 18 abril 2008

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 44 a 84	23 Octubre 2009
Reivindicaciones:	Folios 85 a 90	23 Octubre 2009
	Folios 150 a 154	22 Julio 2011
Dibujos:	Folios 92 a 100	23 Octubre 2009
Prioridad:	US 60/925.716	23 abril 2007

2. Análisis de la Prioridad

Se acepta la reivindicación de Prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486), y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

Título de la solicitud: "Compuestos de estroncio que contienen composiciones de fibrina"

El problema planteado en esta solicitud consiste en la necesidad de proporcionar composiciones para ayudar en la formación de hueso.

Para resolver este problema técnico la solicitud en estudio proporciona una composición para cicatrización ósea y crecimiento óseo en donde la composición comprende fibrina, trombina y un compuesto que contiene estroncio.

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A61L 024/00, A61L 27/22, A61P 19/08;

4. Reivindicaciones relativas a un uso o a un segundo uso (Art 14 y 21 D 486)

El uso de las reivindicaciones 24 a 32 no es patentable, de acuerdo con el Art 14.

5. De la Solicitud de Patente

Titulo

El título no corresponde con el objeto de la solicitud la solicitud hace referencia a composiciones de al menos tres componentes como son fibrinógeno, trombina y un compuesto de estroncio, por lo cual se sugiere al Solicitante hacer la corrección necesaria.

Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 1 no es clara y concisa porque:

- No solo debe caracterizarse por sus componentes de manera general, sino también con las proporciones de estos.
- Se deben establecer inequívocamente los componentes como es el caso del compuesto de estroncio el cual no es definido y no es posible establecer su naturaleza química.
- No es adecuado caracterizar una composición por su uso.

De acuerdo con el capítulo descriptivo la invención esta dirigida a composiciones que además de tener como componentes principales fibrinógeno, trombina y

En todo el capítulo reivindicativo se utilizan los términos generales "compuesto que contiene estroncio" "microparticulado soluble", "proteínas asociadas a células" "proteínas derivadas de plasma", "proteasas", "inhibidores de proteasa", "coligando estroncio", etc., los cuales no son claros y no permite limitar el alcance de la invención puesto que hacen referencia a grupos funcionales o grupos farmacológicos que involucran un numero variado de compuestos con características químicas variadas. Se sugiere al solicitante establecer de manera inequívoca los componentes que hacen parte de la composición sin hacer referencia a nombres generales.

Las reivindicaciones 11 y 17 caracterizan la composición por un paso de transformación o mezcla de uno de sus componentes. Se recuerda al solicitante que tanto los componentes como la composición deben definirse por sí misma (estructura química, componentes y proporciones) y no debe hacerse referencia a procesos o pasos de elaboración. Se sugiere suprimir dichas reivindicaciones o hacer las aclaraciones respectivas.

Las reivindicaciones 10, 14 y 15 caracterizan la composición por la forma como se encuentra uno de sus componentes lo cual no es adecuado puesto que esto no hace parte de las características técnicas esenciales de la composición. Se sugiere establecer las características de dicho componente como característica secundaria dentro de la reivindicación principal, teniendo en cuenta que se debe definir inequívocamente el tipo de compuesto que contiene estroncio dentro de la composición.

Las reivindicaciones 6 y 23 hacen referencia a la forma de administración ó suministro de la composición reclamada, lo cual no la caracteriza correctamente

puesto que hace referencia a la forma de usarla, se sugiere eliminar dichas reivindicaciones.

La reivindicación 8 caracteriza la composición por el hecho de que esta se absorbe y se pone en contacto con un tejido. Lo anterior corresponde a un resultado a alcanzar puesto que no solo sólo la solución propuesta por el solicitante, sino todas las alternativas presentes o futuras que lleguen a ese resultado. Se sugiere reemplazarlo por las características esenciales, estructurales o funcionales, que contribuyen a la obtención de ese efecto.

De acuerdo a lo divulgado en el capítulo descriptivo en las realizaciones preferidas, una característica esencia de la invención consiste en la combinación de sales de estroncio con sales de calcio (fosfato de calcio), por tal razón se sugiere incluir dicha característica dentro de la reivindicación principal.

El capítulo reivindicatorio hace referencia a composiciones que comprenden además de trombina, fibrinógeno y un compuesto de estroncio otros componentes adicionales (plastificante de yodo, proteínas adicionales, coligandos, etc) sin embargo no se encuentra sustento para composiciones que contengan dichos componentes. Se sugiere limitar el alcance de la invención de acuerdo con las realizaciones prefereidas divulgadas en el capítulo descriptivo.

Se presenta una inconsistencia entre la reivindicación 9 y lo establecido por el solicitante en el capítulo descriptivo (folio 56), puesto que los componentes o agentes que inducen coagulación no son agentes adicionales sino por el contrario son alternativas a la trombina.

Descripción (Art 28 D 486)

La Descripción no es clara porque la información que contiene no permite que el la persona del oficio normalmente versada en la materia pueda desarrollar y reproducir la invención en cuanto a la generación de composiciones que contienen mas de los 4 componente (trombina, fibrinógeno, sales de calcio y compuestos de estroncio). Se requiere al Solicitante hacer las aclaraciones necesarias.

6. Determinación del Estado de la Técnica

El Estado de la Técnica se determinó con base en la fecha de presentación de la solicitud de prioridad (23 abril 2007) y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Item	Documento	Título	Fecha de Publicación
D1	US 2001016646	Osteogenic devices and methods of use thereof for repair of endochondral bone, osteochondral and chondral defects	23 agosto 2001
D2	US 200410241314	Strontium-substituted apatite coating	02 diciembre 2004
D3	<u>Biomaterials</u> Volume 28, Issue 2, Pags 3171–3181	The effect of strontium incorporation into CaSiO ₃ ceramics on their physical and biological properties	05 abril 2007

D1 divulga dispositivos osteogénicos y métodos para la reparación de huesos y defectos del cartilago, dichos dispositivos contienen composiciones que promueven la formación acelerada de tejido.

URL: http://worldwide.espacenet.com/publicationDetails/biblio?DB=EPODOC&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20010823&CC=US&NR=2001016646A1&KC=A1

D2 divulga método para preparar una composición implantable con un agente bioactivo de la unión estroncio- apatita. Dicha composición que comprende (i) los iones estroncio, (ii) los iones de calcio, (iii) los iones de fosfato, y (iv) un vehículo líquido.

URL: http://worldwide.espacenet.com/publicationDetails/biblio?CC=US&NR=2004241314A1&KC=A1&FT=D&ND=3&date=20041202&DB=EPODOC&locale=en_EP

D3 divulga el efecto de la combinación de sales de calcio con sales de estroncio en la regeneración ósea (Folios 164-168)

7. Examen de Patentabilidad (Art 14 D486)

Nivel Inventivo (Art18 D486)

La reivindicación 1 consiste en “una composición que comprende fibrinógeno, trombina y un compuesto que contiene estroncio para uso en cicatrización ósea o crecimiento óseo”.

Frente a D1 y D2

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1	D2
Composición para uso en cicatrización ósea o crecimiento óseo	Composición para uso en cicatrización ósea o crecimiento óseo	Composición para uso en crecimiento óseo
fibrinógeno	fibrinógeno	No menciona
trombina	trombina	No menciona
compuesto que contiene estroncio	Sales de calcio	Combinación estroncio con sales de calcio

Los documentos D1 y D2 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 1 porque:

D1 divulga composiciones y métodos para el tratamiento de defectos en cartilago y hueso (resumen). Dichas composiciones comprenden trombina, fibrinógeno y fosfato de tri-calcio (párrafo 0129) las cuales se reabsorben y mejoran la formación de hueso luego de su implantación (párrafo 0124-0131)

Asimismo, se divulga que la combinación de fibrinógeno y trombina tiene características de agente gelificante (párrafo 0074-0075) y que en presencia del una matriz de calcio (fosfato) forman una masilla moldeable de tipo gel (párrafo 0120 - 0121) que puede inyectarse a la zona deseada (reivindicación 40). Por otro lado se divulga que las partículas de calcio tienen una dimensión de partícula de 50-2000um (párrafo 0129 reivindicaciones 14 y 15).

Adicionalmente dichas composiciones pueden contener proteínas extracelulares como son fibronectina, factores de coagulación (párrafo 0122) y proteínas morfo génicas (reivindicación 21) y agentes de tipo plastificante como son polialcoholes, polisacáridos, etc.

La diferencia entre la invención, definida en la reivindicación 1 y la composición divulgada en D1 consiste en la presencia de un compuesto que contiene estroncio adicionalmente a los componentes fibrinógeno, trombina y sales de calcio.

El efecto que logra el incluir la presencia de un compuesto de estroncio dentro de las composiciones que contienen fibrinógeno, trombina y sales de calcio, es que se incrementa la resistencia mecánica del hueso.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: ¿cómo modificar las composiciones divulgadas en D1 para el manejo de lesiones y defectos óseos para conseguir un incremento en la resistencia mecánica del hueso generado?

Por su lado el documento D2 divulga composiciones para mejorar los defectos óseos y método para preparar una composición implantable con un agente bioactivo obtenido por la unión estroncio- apatita. Dicha composición que comprende (i) los iones estroncio, (ii) los iones de calcio, (iii) los iones de fosfato, y (iv) un vehículo líquido (resumen, reivindicación 1) tiene como efecto principal ser agente mejorador de la formación de hueso (columna 4 líneas 18-24) y puede combinarse con agentes biológicos que incluyen factores de crecimiento, proteínas de matriz extracelular (columna 6, líneas 12 a 19) y agentes calcimiméticos como la osteocalcina (columna 6, línea 22).

De igual manera, D2 divulga la combinación de cloruro de estroncio con apatita (sales de calcio) luego de la mezcla de estos dos componentes de tal manera que se forma sal de los dos compuestos con una relación entre estroncio y calcio 1:3 o 1:10 (columna 4, líneas 28-29; reivindicación 1 y 16). Dentro de la composición la concentración de iones estroncio se encuentra entre 0.5nM y 5mM (columna 5, líneas 25-27, reivindicación 2), por lo que la cantidad de estroncio, calcio e iones fosfato dentro de la composición determina la funcionalidad y la resistencia de la cubierta dentro del implante (ejemplo 1). Si bien es cierto que el documento D2 no divulga una matriz gelificable, si establece la pertinencia de la mezcla de estroncio y calcio para mejorar la resistencia y aplicabilidad del implante óseo para animales, humanos, etc.

En consecuencia, la persona del oficio normalmente versada en la materia, que pretenda obtener composiciones para uso en cicatrización ósea o crecimiento óseo que conozca el estado de la técnica, estaría motivada a incluir la mezcla entre estroncio y calcio, como agentes que dan mayor resistencia mecánica al hueso, divulgada en el documento D2, dentro de las composiciones divulgadas en el documento D1 para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia.

Puesto que las reivindicaciones dependientes 2, 3, 6 a 23 no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

Frente a D1 y D3

Comparación de las características técnicas **esenciales**, con las del Estado de la Técnica:

Características esenciales	D1		D3
Composición para uso en cicatrización ósea o crecimiento óseo	Composición crecimiento óseo	para	No menciona
fibrinógeno	fibrinógeno		No menciona
trombina	trombina		No menciona
compuesto que contiene estroncio	Sales de calcio		Combinación estroncio con sales de calcio

Los documentos D1 y D3 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 1 porque:

D1 divulga composiciones y métodos para el tratamiento de defectos en cartílago y hueso (resumen). Dichas composiciones comprenden trombina, fibrinógeno y fosfato de tri-calcio (párrafo 0129) las cuales se reabsorben y mejoran la formación de hueso luego de su implantación (párrafo 0124-0131)

Asimismo, se divulga que la combinación de fibrinógeno y trombina tiene características de agente gelificante (párrafo 0074-0075) y que en presencia de una matriz de calcio (fosfato) forman una masilla moldeable de tipo gel (párrafo 0120 - 0121) que puede inyectarse a la zona deseada (reivindicación 40). Por otro lado se divulga que las partículas de calcio tienen una dimensión de partícula de 50-2000um (párrafo 0129 reivindicaciones 14 y 15).

Adicionalmente dichas composiciones pueden contener proteínas extracelulares como son fibronectina, factores de coagulación (párrafo 0122) y proteínas morfo génicas (reivindicación 21).

La diferencia entre la invención, definida en la reivindicación 1 y la composición divulgada en D1 consiste en la presencia de un compuesto que contiene estroncio adicionalmente a los componentes fibrinógeno, trombina y sales de calcio.

El efecto que logra el incluir la presencia de un compuesto de estroncio dentro de las composiciones que contienen fibrinógeno, trombina y sales de calcio, es que se incrementa la resistencia mecánica del hueso.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: ¿cómo modificar las composiciones divulgadas en D1 para el manejo de lesiones y defectos óseos para conseguir un incremento en la resistencia mecánica del hueso generado?

Sin embargo el documento D3 da solución a dicho problema técnico pues divulga estudios que demuestran la pertinencia y el uso de sales combinadas de estroncio y calcio (sales de Sr-CaSiO_3) como material bioactivo para la regeneración ósea, incremento de la actividad de osteoblastos y la estimulación de tejido óseo (Folio 164, Ítem 1). Asimismo, se divulga que el aumento en la concentración de iones estroncio genera un aumento en la densidad de las sales de calcio lo que lleva a una mayor resistencia del hueso (osteocitos) en las pruebas experimentales y a la disminución de la degradación y disolución de las sales de calcio (Folio 166, ítem 4; folio 168, ítem 5)

La reivindicación es obvia, porque la persona del oficio normalmente versada en la materia enfrentada al problema de cómo modificar las composiciones divulgadas en D1 para el manejo de lesiones y defectos óseos para conseguir un incremento en la resistencia mecánica del hueso generado?, incluiría sales combinadas de estroncio y calcio (sales de Sr-CaSiO_3) como material bioactivo para la regeneración ósea que se revelan en D3, dentro de las composiciones que se enseñan en D1, logrando de esta forma la composición objeto de esta solicitud.

Por lo cual la composición, de la Reivindicación 1 carece de Nivel Inventivo.

Puesto que las reivindicaciones dependientes 2, 6 a 23 no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

8. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Item	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	US 2001016646	2, 6 a 23	Nivel Inventivo
D2	US 200410241314	2, 6 a 23	Nivel Inventivo
D3	<u>Biomaterials</u> Volume 28, Issue 2, Pags 3171-3181	2, 6 a 23	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante y presentarla por escrito

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha 13 AGO. 2012

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The effect of strontium incorporation into CaSiO_3 ceramics on their physical and biological properties

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Abstract

Cs-510® separates phase from material, as an inherent bioactive material, the bone regeneration of **Cs-510**® (Sr-apatite-cement) is mainly based on the inherent effects on bone formation. The aim of this study was to incorporate Sr into **Cs-510**® to improve its bone-forming ability, bone formation, osteo-inductive property, osteo-conduction property, osteo-formation ability, bone formation, and to investigate their effects on phase transition, maturation, and ability of aqueous formation in **SrHf**. The ionic dissolution rate of the Sr-**Cs-510**® and human bone-derived cells (HBDC) proliferation of **Cs-510**® containing **Cs-510**® (Sr-**Cs-510**®) cements at various concentrations (0–10% Sr) were prepared. The incorporation of Sr into **Cs-510**® promoted the phase transition from α to β phase, and the **Cs-510**® contained cements demonstrated that they did not alter the mechanism and ability of aqueous formation in **SrHf**. The ionic dissolution rate of the Sr-**Cs-510**® decreased compared to the **Cs-510**®. The addition of Sr decreased pH value in **SrHf**. The effect of Sr-**Cs-510**® extracts, carried out according to the International Standard Organization, on HBDC proliferation was evaluated. At high extract concentration (100 and 1000 µg/ml), **Cs-510**® was found to stimulate HBDC proliferation, however, the incorporation of Sr into **Cs-510**® stimulated HBDC proliferation even at low extract concentration (ranging from 12.5–25 to 50 mg/ml). Our results indicate that Sr-**Cs-510**® cements improved the physical and biological properties of the pure **Cs-510**® cements.

Get ready, Set, Go! Phase Transition, Nucleation ability, Dissolution, Proliferation

Interpretation

CaSiO₃ ceramics are regarded as potential bioactive materials for bone tissue regeneration due to their bioactive properties [1]. A major drawback of the CaSiO₃ ceramics is their high dissolution and degradation rate leading to a high pH value in the surrounding environment [5,6] which can disadvantage cell growth thereby affecting their osteoinductive ability [2]. Strongly acidic environment is used for treatment of osteoporosis [8,9]. It was found to induce osteoclast activity [10–14] by stimulating bone formation and reducing bone resorption [1,15,16]. It has great affinity to bone [17] and is incorporated into it by two mechanisms of surface exchange involving the incorporation of Si into

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2. Materials and methods

100

5% CaSiO₃ powders containing 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0% Si were synthesized by chemical precipitation method according to our previous work.¹¹ The 5% CaSiO₃ powders were sieved to 250 meshes and then dispersed in a 10% aqueous solution of dimethylacrylamide (DMAA). Each the prepared colloidal emulsion disks, 5% CaSiO₃ powders were mixed with 6 wt% polyethylene glycol (PEG) 400. The emulsion disks were solution casted on glass slides with a thickness of 100 μ m. The mixture was initially pressed at 10 MPa volume pressure to get 5% CaSiO₃ gel emulsion disks with a dimension of 0.5 $\times$ 2 cm. Subsequently, the green disks were sintered at 1100 and 1250 $^{\circ}$ C for 1h with a heating rate of 5 $^{\circ}$ C/min to obtain the ceramic disks.

The selected Sr-Ca/SrO, ceramids, disks were analyzed using X-ray fluorescence (XRF), Inductively coupled plasma atomic emission spectroscopy (ICP-AES), Philips N1, 30 °C, The Netherlands). The apparent density D of the ceramids was measured according to Archimedes' principle using the following formulation: $D = M_1/(M_2 - M_3)$, where M_1 is the weight of sample in air, M_2 the weight of sample with water, and M_3 the weight of sample in water. The relative density Rd was calculated using the formulation $Rd = D/\rho_{theor}$, where D is the apparent density of ceramids and ρ_{theor} the theoretical density of the materials.

The stability of Sr-CaSiO_3 ceramics to form an apatite in vivo

SIM containing an concentration similar to those in human blood. The solutions were prepared according to the method described by Kolb and Schmitt, respectively, CaCl_2 , H_2PO_4 , NaHCO_3 , NaCl , KCl , MgSO_4 , Na_2SO_4 , NaH_2PO_4 , and $\text{Na}_2\text{S}_2\text{O}_8$ were dissolved in distilled water and adjusted to 1.4, 1.8, 2.5, 3.5, 4.5, 5.5, 6.5, 7.5, 8.5, 9.5, 10.5, 11.5, 12.5, 13.5, 14.5, 15.5, 16.5, 17.5, 18.5, 19.5, 20.5, 21.5, 22.5, 23.5, 24.5, 25.5, 26.5, 27.5, 28.5, 29.5, 30.5, 31.5, 32.5, 33.5, 34.5, 35.5, 36.5, 37.5, 38.5, 39.5, 40.5, 41.5, 42.5, 43.5, 44.5, 45.5, 46.5, 47.5, 48.5, 49.5, 50.5, 51.5, 52.5, 53.5, 54.5, 55.5, 56.5, 57.5, 58.5, 59.5, 60.5, 61.5, 62.5, 63.5, 64.5, 65.5, 66.5, 67.5, 68.5, 69.5, 70.5, 71.5, 72.5, 73.5, 74.5, 75.5, 76.5, 77.5, 78.5, 79.5, 80.5, 81.5, 82.5, 83.5, 84.5, 85.5, 86.5, 87.5, 88.5, 89.5, 90.5, 91.5, 92.5, 93.5, 94.5, 95.5, 96.5, 97.5, 98.5, 99.5, 100.5, 101.5, 102.5, 103.5, 104.5, 105.5, 106.5, 107.5, 108.5, 109.5, 110.5, 111.5, 112.5, 113.5, 114.5, 115.5, 116.5, 117.5, 118.5, 119.5, 120.5, 121.5, 122.5, 123.5, 124.5, 125.5, 126.5, 127.5, 128.5, 129.5, 130.5, 131.5, 132.5, 133.5, 134.5, 135.5, 136.5, 137.5, 138.5, 139.5, 140.5, 141.5, 142.5, 143.5, 144.5, 145.5, 146.5, 147.5, 148.5, 149.5, 150.5, 151.5, 152.5, 153.5, 154.5, 155.5, 156.5, 157.5, 158.5, 159.5, 160.5, 161.5, 162.5, 163.5, 164.5, 165.5, 166.5, 167.5, 168.5, 169.5, 170.5, 171.5, 172.5, 173.5, 174.5, 175.5, 176.5, 177.5, 178.5, 179.5, 180.5, 181.5, 182.5, 183.5, 184.5, 185.5, 186.5, 187.5, 188.5, 189.5, 190.5, 191.5, 192.5, 193.5, 194.5, 195.5, 196.5, 197.5, 198.5, 199.5, 200.5, 201.5, 202.5, 203.5, 204.5, 205.5, 206.5, 207.5, 208.5, 209.5, 210.5, 211.5, 212.5, 213.5, 214.5, 215.5, 216.5, 217.5, 218.5, 219.5, 220.5, 221.5, 222.5, 223.5, 224.5, 225.5, 226.5, 227.5, 228.5, 229.5, 230.5, 231.5, 232.5, 233.5, 234.5, 235.5, 236.5, 237.5, 238.5, 239.5, 240.5, 241.5, 242.5, 243.5, 244.5, 245.5, 246.5, 247.5, 248.5, 249.5, 250.5, 251.5, 252.5, 253.5, 254.5, 255.5, 256.5, 257.5, 258.5, 259.5, 260.5, 261.5, 262.5, 263.5, 264.5, 265.5, 266.5, 267.5, 268.5, 269.5, 270.5, 271.5, 272.5, 273.5, 274.5, 275.5, 276.5, 277.5, 278.5, 279.5, 280.5, 281.5, 282.5, 283.5, 284.5, 285.5, 286.5, 287.5, 288.5, 289.5, 290.5, 291.5, 292.5, 293.5, 294.5, 295.5, 296.5, 297.5, 298.5, 299.5, 300.5, 301.5, 302.5, 303.5, 304.5, 305.5, 306.5, 307.5, 308.5, 309.5, 310.5, 311.5, 312.5, 313.5, 314.5, 315.5, 316.5, 317.5, 318.5, 319.5, 320.5, 321.5, 322.5, 323.5, 324.5, 325.5, 326.5, 327.5, 328.5, 329.5, 330.5, 331.5, 332.5, 333.5, 334.5, 335.5, 336.5, 337.5, 338.5, 339.5, 340.5, 341.5, 342.5, 343.5, 344.5, 345.5, 346.5, 347.5, 348.5, 349.5, 350.5, 351.5, 352.5, 353.5, 354.5, 355.5, 356.5, 357.5, 358.5, 359.5, 360.5, 361.5, 362.5, 363.5, 364.5, 365.5, 366.5, 367.5, 368.5, 369.5, 370.5, 371.5, 372.5, 373.5, 374.5, 375.5, 376.5, 377.5, 378.5, 379.5, 380.5, 381.5, 382.5, 383.5, 384.5, 385.5, 386.5, 387.5, 388.5, 389.5, 390.5, 391.5, 392.5, 393.5, 394.5, 395.5, 396.5, 397.5, 398.5, 399.5, 400.5, 401.5, 402.5, 403.5, 404.5, 405.5, 406.5, 407.5, 408.5, 409.5, 410.5, 411.5, 412.5, 413.5, 414.5, 415.5, 416.5, 417.5, 418.5, 419.5, 420.5, 421.5, 422.5, 423.5, 424.5, 425.5, 426.5, 427.5, 428.5, 429.5, 430.5, 431.5, 432.5, 433.5, 434.5, 435.5, 436.5, 437.5, 438.5, 439.5, 440.5, 441.5, 442.5, 443.5, 444.5, 445.5, 446.5, 447.5, 448.5, 449.5, 450.5, 451.5, 452.5, 453.5, 454.5, 455.5, 456.5, 457.5, 458.5, 459.5, 460.5, 461.5, 462.5, 463.5, 464.5, 465.5, 466.5, 467.5, 468.5, 469.5, 470.5, 471.5, 472.5, 473.5, 474.5, 475.5, 476.5, 477.5, 478.5, 479.5, 480.5, 481.5, 482.5, 483.5, 484.5, 485.5, 486.5, 487.5, 488.5, 489.5, 490.5, 491.5, 492.5, 493.5, 494.5, 495.5, 496.5, 497.5, 498.5, 499.5, 500.5, 501.5, 502.5, 503.5, 504.5, 505.5, 506.5, 507.5, 508.5, 509.5, 510.5, 511.5, 512.5, 513.5, 514.5, 515.5, 516.5, 517.5, 518.5, 519.5, 520.5, 521.5, 522.5, 523.5, 524.5, 525.5, 526.5, 527.5, 528.5, 529.5, 530.5, 531.5, 532.5, 533.5, 534.5, 535.5, 536.5, 537.5, 538.5, 539.5, 540.5, 541.5, 542.5, 543.5, 544.5, 545.5, 546.5, 547.5, 548.5, 549.5, 550.5, 551.5, 552.5, 553.5, 554.5, 555.5, 556.5, 557.5, 558.5, 559.5, 560.5, 561.5, 562.5, 563.5, 564.5, 565.5, 566.5, 567.5, 568.5, 569.5, 570.5, 571.5, 572.5, 573.5, 574.5, 575.5,

3.2 Dissolution rate of Sr-Ca-SiO₃ ceramics in SBF

The changes in concentrations of Ca^{2+} , Sr^{2+} and $\text{Sr}^{2+}/\text{Ca}^{2+}$ after leaching were measured by inductively coupled plasma atomic emission spectrometry (ICP-AES, Perkin Elmer, Optima 4000D, USA), and the concentrations in pH value of the solution were determined on a colorimetric pH meter. Based on the fact that there is no Sr^{2+} in SHF before washing, the dissolution ratio of the Sr-Ca-MSO examined at different time periods can be calculated by the following equation: $X = (a - b) / (a - c)$, where a , b and c represent the Sr concentration in SHF (mg/L) at 0, t and ∞ respectively, and X is the dissolution ratio of the samples washed in SHF, respectively.

Human bone-marrow cells (HBC) in isolation and culture

[illegible]

prepared and used for the proliferation assay. Permission to use discarded human tissue was granted by the Human Ethics Committee of the University of Sydney.

25. The effect of Sr containing tonic products from Sr-CaSiO₃ on

The Sr-CaSO_4 powder extract was diluted in full culture medium according to International Standard Organization (ISO) 10993-5 [14]. The dissolution extracts of ceramics were prepared by adding Sr-CaSO_4 powders to serum free α -MEM culture medium at a ratio of 20 mg/ cm^2 powder to medium. After incubation at 37°C for 24 h, the mixture was centrifuged and the supernatant was collected. Serial dilutions of extracts (100, 50, 25, 12.5, and 6.25 mg/L) were prepared using serum-free α -MEM medium without β -glutamine or ascorbic acid. Subsequently, the diluted extracts were sterilized using 0.22 μm filter and used in THXPC cell differentiation experiments. The ionic concentration of the diluted extracts was measured by ICP-AES (Table 1).

THPC was seeded at a density of 1×10^5 cells/well into 96 well plates and incubated for 24 h. The culture medium was then removed and replaced by 50 μ L of α -MEM medium supplemented with 20% FCS. The culture medium was then replaced by 50 μ L of α -MEM medium supplemented with 20% FCS without addition of eluted extracts were used as a blank control (blank), and 50 μ L solution of 0.2% Triton X-100 and 50 μ L of α -MEM medium supplemented with 20% FCS were used as a negative control (0-tr). Cells were then incubated at 37 °C in 5% CO₂ for 1, 3, and 7 d.

Variable cell numbers were evaluated at appropriate time intervals (1, 3, 5, 7, and 7 d) using MTS assay (Promega, Madison, WI, USA) according to the manufacturer's instructions. The MTS (tetrazolium) compound is a water-soluble version of 3-(4,5-dimethylthiazol-2-yl)-5-phenyltetrazolium bromide (MTS). It is reduced by cells into a colored formazan product which is soluble in culture medium. The quantity of formazan product is directly proportional to the number of living cells in culture.

At the predetermined time point, 100 μ l of 0.2 mg/mL MTS solution was added in each well and was incubated for 4 h at 37 °C to take up the dye. The reaction was stopped by the addition of dimethyl sulfoxide (DMSO). The absorbance of the formazan was read at 490 nm using an ELISA plate reader and a software: AccuFit/MTS. Result was expressed as absorbance reading from each well minus the optical density value of blank wells.

5.3.3.3. *Significance analysis*

Results were expressed as means $\pm$ standard deviation (SD) for all experiments and were analyzed using one-way ANOVA with a post-hoc test. A *p*-value ≤ 0.05 was considered statistically significant.

5. Results

1. Characterization of Sr-CaSiO₃ ceramics *

The incorporation of Sr into CaSiO_3 ceramics resulted in a phase transition of CaSiO_3 from β to α as shown in the NRD results in Fig. 1. The transition was more prominent with increased Sr concentration. At 1% Sr content, α - CaSiO_3 phase was obtained, while at 2.5% Sr there was a mixed composition of both α - CaSiO_3 and β - CaSiO_3 . At 5% Sr content, only α -phases were present. DTA analysis showed that with an increase in Sr content, the temperature of phase transition from β to α phase decreased (Fig. 2). The NRD patterns of the Sr- CaSiO_3 ceramics sintered at 1250°C are shown in Fig. 3. Only α - CaSiO_3 peaks (standard modes) were

Table 1
The Ca, Sr and Sr ion concentrations of Sr-CaSiO₃ extracts (mmol/L)

Materials	Ions	Extract concentrations (mg/mL)				
		Blank	6.25	12.5	25	50
CaSiO ₃	Ca	2.43	1.47	1.55	1.81	3.12
	Sr	0.09	0.09	0.18	0.25	1.15
	Sr	0	0	0	0	0
Sr 1%–CaSiO ₃	Ca	2.43	1.42	1.55	1.81	2.32
	Sr	0	0.05	0.10	0.19	0.35
	Sr	0	0.002	0.006	0.006	0.025
Sr 2.5%–CaSiO ₃	Ca	2.43	1.45	1.61	1.95	3.56
	Sr	0	0.07	0.13	0.27	0.53
	Sr	0	0.003	0.007	0.013	0.027
Sr 5%–CaSiO ₃	Ca	2.43	1.41	1.53	1.78	2.77
	Sr	0	0.05	0.10	0.20	0.39
	Sr	0	0.007	0.015	0.030	0.059
Sr 10%–CaSiO ₃	Ca	2.43	1.44	1.59	1.89	2.49
	Sr	0	0.07	0.14	0.29	0.57
	Sr	0	0.014	0.028	0.057	0.114

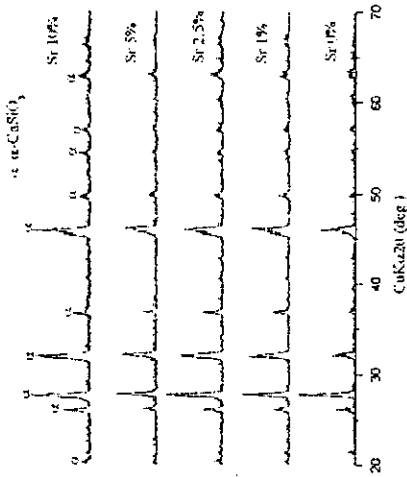


Fig. 3. XRD patterns of Sr-CaSiO₃ ceramics sintered at 1250 °C.

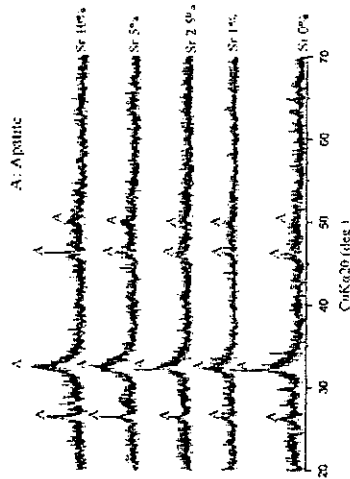


Fig. 5. XRD patterns of Sr-CaSiO₃ ceramics after soaking in SBF for 14 d.

release of Sr ions concentrations in SBF and the Sr contents in Sr-CaSiO₃ ceramics (Fig. 8(c)). The weight loss of Sr-CaSiO₃ ceramics was calculated according to the Si concentrations in SBF and was found to increase with an increase of soaking time (Fig. 8(d)). Furthermore, the Sr containing CaSiO₃ ceramics decreased the weight loss, compared to CaSiO₃ containing 2.5% Sr resulted in the lowest weight loss (Fig. 8(d)). pH value of Sr containing CaSiO₃ ceramics-soaked solution was significantly lower compared to CaSiO₃ ceramics (Fig. 9). The pH value increased in the first 6 d of soaking the disks (both modified and unmodified with Sr) in SBF, followed by a decrease at 7 and 8 d of soaking and this trend reversed (increased) at 9 d of soaking. Interestingly, the pH values of CaSiO₃ containing 5% and 10% Sr are comparable but lower than those of CaSiO₃ containing 1% and 2.5% Sr (Fig. 9).

3.4. The effect of Sr-CaSiO₃ extracts on HBDC proliferation

The effect of ions released from Sr-CaSiO₃ at different concentrations on HBDC proliferation was evaluated at 1, 3, and 7 d. HBDC proliferation was comparable when cells were cultured with the different concentration of extracts for 1 and 3 d (Fig. 10). However, at day 7, a significant increase in HBDC proliferation was observed (Fig. 10). CaSiO₃ with no Sr stimulated cell proliferation at extract concentration range (0–200 mg/mL) (Fig. 10(a)) compared to the blank control and had no stimulatory effect on cell proliferation at lower extract concentration range (12.5–50 mg/mL). However, with the incorporation of Sr into CaSiO₃, HBDC proliferation increased significantly even at lower extract concentration range (12.5–50 mg/mL), compared to CaSiO₃ (Figs. 10(b)–(e)).

Ionic concentrations of Ca, Sr and Sr increased with an increase in extract concentrations (Table 1). When extracts of CaSiO₃ were added to the culture, only high concentration

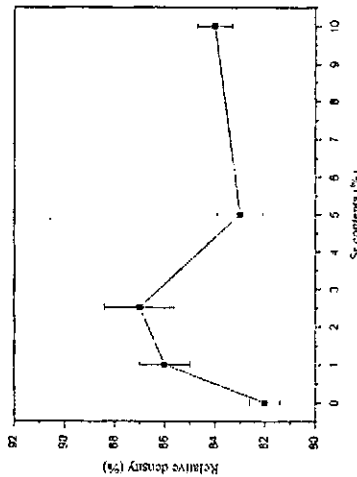


Fig. 4. The effect of Sr contents on the relative density of CaSiO₃ ceramics.

ceramics after soaking in SBF for 14 d are shown in Fig. 7. Silica-rich layer was formed on the different Sr containing CaSiO₃ ceramics with an apatite layer deposited on the silica-rich layer. The thickness of the formed apatite on different Sr containing CaSiO₃ ceramics was similar and varied within the range of 20–30 μm.

3.5. Ion release, pH values, and apatite layer of Sr-CaSiO₃ ceramics in SBF

The ion release of Sr-CaSiO₃ ceramics in SBF solution is shown in Fig. 8. The concentrations of Ca and Sr ions in pure CaSiO₃ soaked in SBF solution were higher than those of Sr containing CaSiO₃. Sr-CaSiO₃ ceramics at 2.5% Sr concentration resulted in the lowest Sr ion release (Fig. 8(b)). A positive correlation existed between the

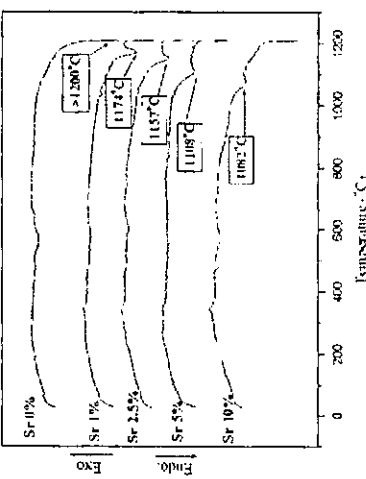


Fig. 2. DTA curves of Sr-CaSiO₃ with different Sr contents.

24–0033) existed in the XRD pattern and there was no difference in the intensity of peaks with different Sr contents in CaSiO₃ ceramics. SEM micrographs showed an obvious apatite layer is formed on the Sr-CaSiO₃ ceramics (Fig. 6). However, their microstructure differed significantly with the different Sr concentrations used (Fig. 6). A smooth apatite layer is formed on pure CaSiO₃ ceramics and the apatite microcrystals are not evident (Fig. 6(a)). When Sr contents of 1% to 2.5% were used, the apatite microcrystals had a lath-like structure where the crystal boundaries were more evident (Figs. 6(b) and (c)). In contrast, ball-like structure of apatite was formed at 5% and 10% Sr contents (Figs. 6(d) and (e)). The SEM images and EDS analysis of the cross-section of Sr-CaSiO₃

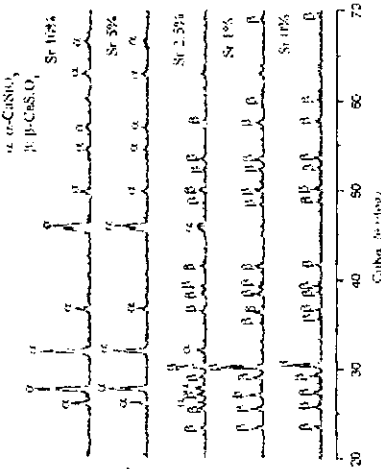


Fig. 1. XRD patterns of Sr-CaSiO₃ ceramics sintered at 1100 °C.

(1–0300) were evident, which indicates that Sr-CaSiO₃ ceramics maintain α -CaSiO₃ structure at Sr concentrations of 1%, 2.5%, 5%, and 10%. The effect of Sr contents on the relative density is shown in Fig. 4, where low Sr contents (1% and 2.5%) significantly enhanced the densification of CaSiO₃ ceramics ($p < 0.05$) and as Sr concentration increased (5% and 10%) no obvious effect was found on the densification.

3.2. Apatite-formation ability of Sr-CaSiO₃ ceramics

The XRD patterns of Sr-CaSiO₃ ceramics after soaking in SBF for 14 d are presented in Fig. 3. Only the characteristic peaks of apatite (Standard cards no JCPD)

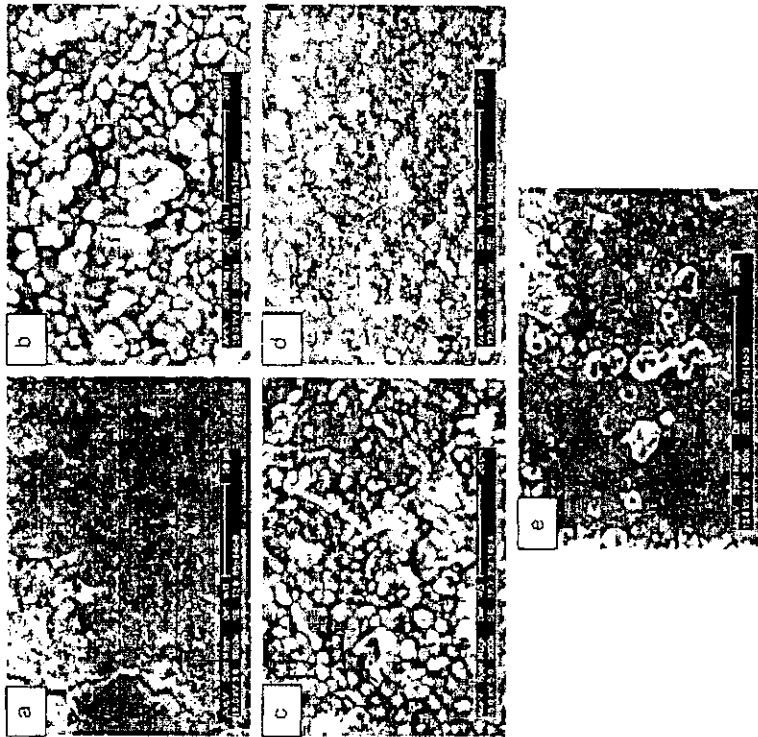


Fig. 6. SPM micrographs of Sr-CaSiO₃ ceramics after sintering in NBF for 14d: (a) Sr 1%, (b) Sr 2.5%, (c) Sr 5%, (d) Sr 10%, and (e) Sr 10%.

range of Ca (3.35–5.49 mol) and Si (1.37–2.70 mol) resulted in enhanced HBDC proliferation compared to the blank control. However, lower concentration of Ca (1.53–2.50 mol) and Si (0.09–0.69 mol) resulted in enhanced HBDC proliferation when Sr was incorporated into the CaSiO₃ (Table 1 and Fig. 10).

Discussion

The results of this study demonstrate that the incorporation of Sr into CaSiO₃ ceramics characterizes phase composition which is regulated by the sintering temperatures used. At 1100 °C, increase of Sr contents resulted in phase transition from β -CaSiO₃ to α -CaSiO₃. In addition, the temperature of phase transition decreased with increase of Sr contents. Previously, it was shown that the incorporation of some foreign ions in TiO₂ can stimulate the phase transition from rutile to anatase, which was attributed to the formation of oxygen vacancies that enhanced the transport of atoms and accelerated phase

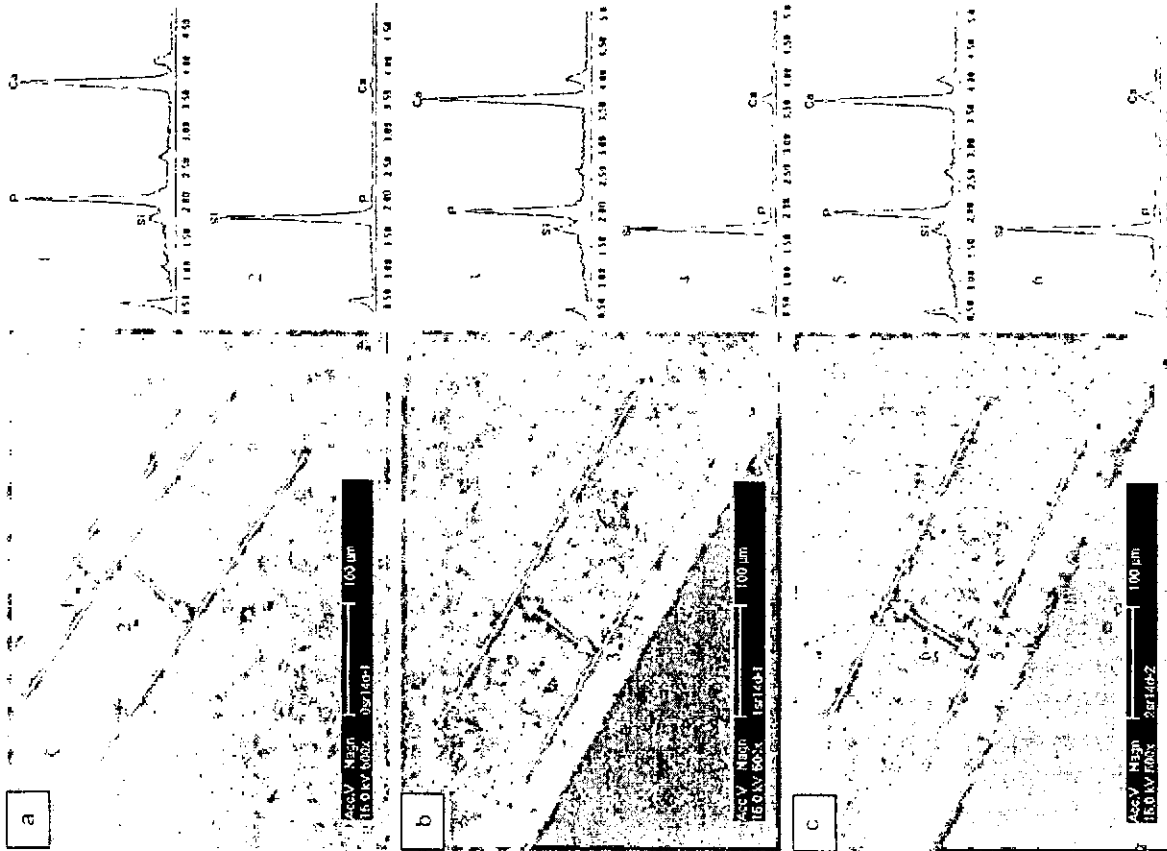
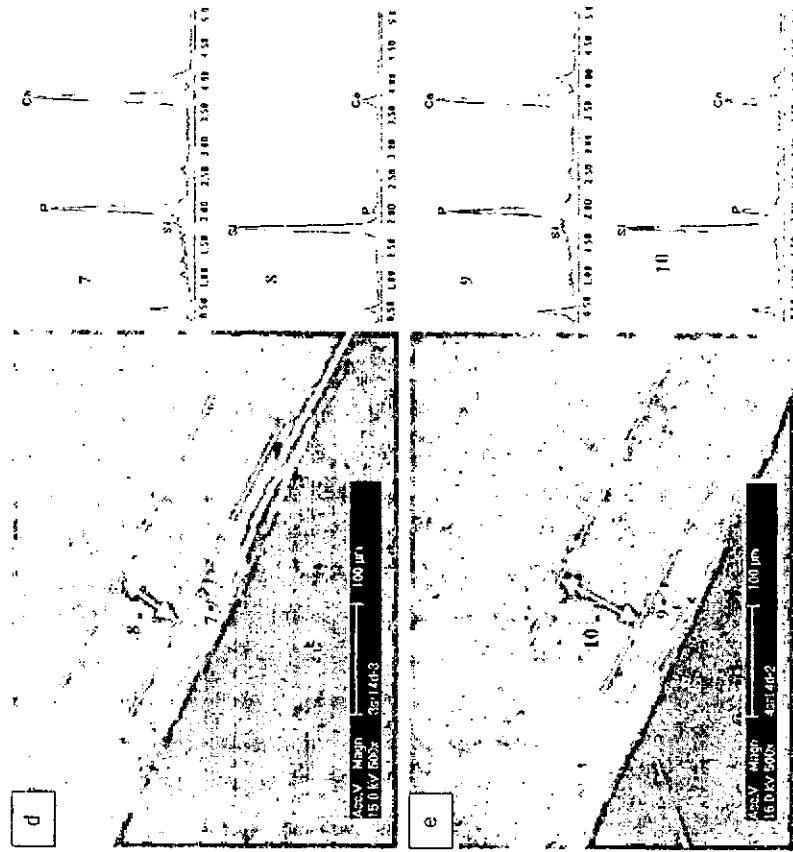
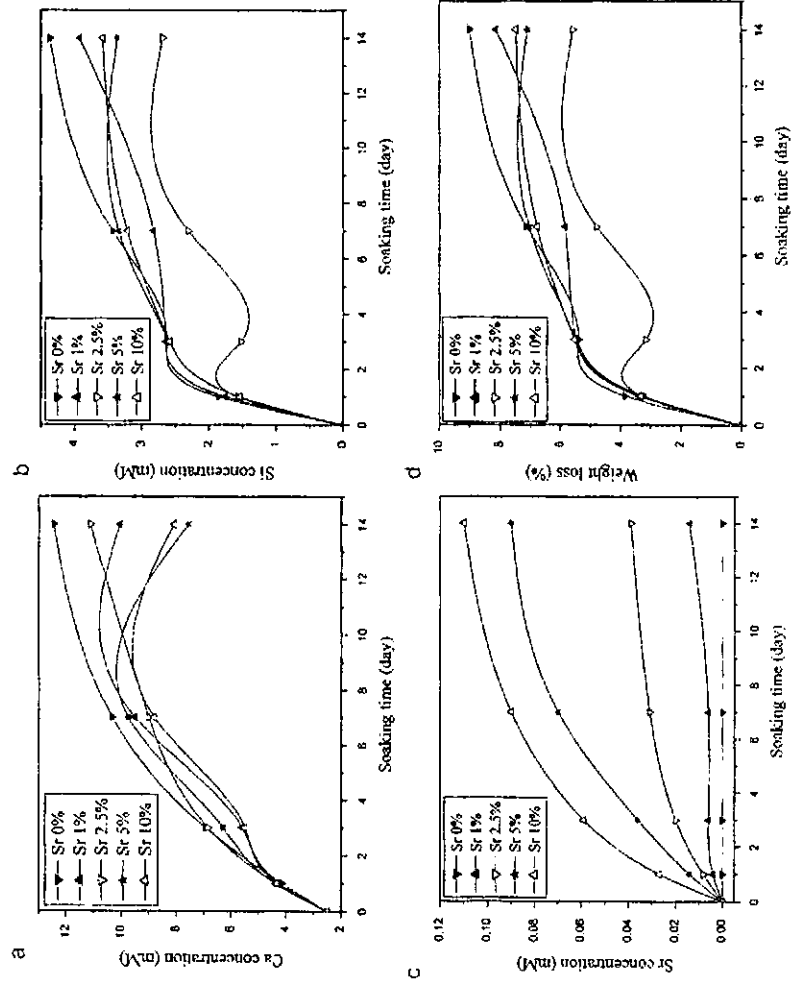
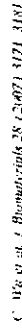
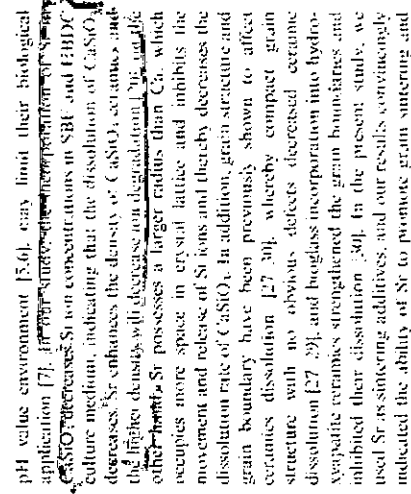


Fig. 7. SPM micrographs and EDX point analysis of the coexistence of Sr-CaSiO₃ ceramics after sintering in NBF for 14d: (a) Sr 1%, (b) Sr 2.5%, (c) Sr 5%, (d) Sr 10%, and (e) Sr 10%.



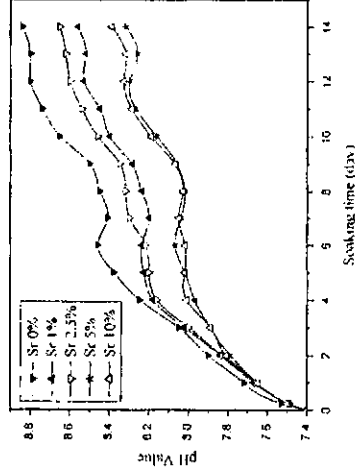
additive promoted the densification of $(\text{Pb}_{0.95}\text{Ti}_{0.05})_{1-x}\text{Sr}_x\text{ZrO}_3$ ceramics through the replacement of Pb^{2+} by Cd^{2+} . Therefore, it is reasonable to speculate that the mechanisms by which Sr induced densification of the CaSiO_3 ceramics in the present study may be related to the fact that Sr ion ($r_{\text{Sr}^{2+}} = 1.18 \text{ \AA}$) possesses a larger radius than Ca ($1.10 \text{ \AA}$) as shown previously, which causes an increase in crystal dimension, thereby enhancing the transport of atoms and accelerates the densification of CaSiO_3 ceramics. However, at 5% and 10% concentration, Sr can cause a further increase in crystal dimension leading to the formation of a loose structure which results in a decreased density compared with those of 1% and 2.5% Sr containing CaSiO_3 ceramics.

CaSiO₃ ceramics have been proposed as potential materials for bone tissue regeneration, since they proved to be bioactive, degradable and biocompatible [1-3]. However, their high degradation rate, leading to a high



strengthen the grain boundary and grain densification, thereby decreasing the dissolution of CaSiO_3 ceramics. The incorporation of Sr into CaSiO_3 decreases the pH value in SBC. The pH value has multiple effects on HBD/C metabolism and function [34]. The most important and interesting is that when bone forms, the crosslinking of the collagen chains and the subsequent precipitation of hydroxyapatite are pH dependent and require an optimally pH at the bone formation site [35]. Our results showed that the incorporation of Sr in CaSiO_3 decreased the pH value in SBC, suggesting a potential role for the incorporation of Sr into bone cells culture.

Our results showed that the release of Sr ions in SBF and cell culture medium can be tailored by varying the Sr concentration in CaSiO_3 . The release of Sr ions into the SBF and cell culture medium was low, where the maximum ratio of Sr/Ca in SBF was no more than $1.5 \text{ mol}\%$, and the maximum ratio of Sr/Ca in cell culture medium was no



plausible to suggest that the potential mechanism of the specific stimulatory effect seen on human bone cells can be accredited to the appropriate combination of Sr, Ca and Si ions.

5. Conclusions

We have successfully developed novel Sr-CaSiO₃ ceramic disks with altered physical and biological activities. The incorporation of Sr promoted phase transition from β -CaSiO₃ to α -CaSiO₃. Sr (1% and 2.5%) incorporation into CaSiO₃ resulted in enhanced densification of the ceramics while higher Sr contents (5% and 7.5%) had no obvious effect on the densification. Moreover, the incorporation of Sr decreased the dissolution of CaSiO₃ ceramics and pH value of SBF and did not change the mechanism and ability of apatite formation in SBF. The incorporation of Sr into CaSiO₃ stimulated HBDC proliferation. Our results indicate that Sr-CaSiO₃ ceramics possess improved physical and biological properties as compared with pure CaSiO₃ ceramics.

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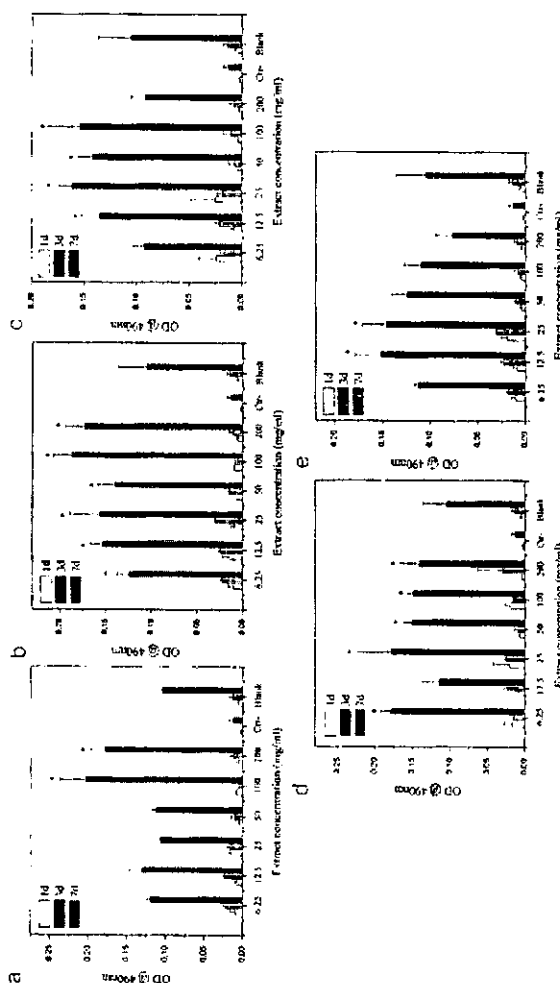


Fig. 10. The effect of Sr-CaSiO₃ extracts with different extract concentrations on osteoblast proliferation. (a) Sr 1% CaSiO₃, (b) Sr 2.5% CaSiO₃, (c) Sr 5% CaSiO₃, and (d) Sr 7.5% CaSiO₃. The corresponding concentrations of Ca, Sr, and Si ions in extracts were listed in Table 1. *The experimental group compared with the blank control group after 7 d of culture, $p < 0.05$. Blank, blank control; Ctrl = negative control.

more than 4 mol%. The normal concentration of Sr in human skeleton is about 3.5 mol% of Ca content [33]. Therefore, the released Sr ion concentration from Sr-CaSiO₃ is similar to the Sr concentration found in human bone.

As already reported by Kokubo and Siriphanont [1,22], fast apatite formation in SBF was found in the presence of a Si-rich layer on the surface of the reacted samples by dissolution of CaSiO₃. The mechanism for this fast apatite formation is thought to occur by selective adsorption of Ca²⁺ to Si OH on the surface of Si-rich layer and subsequent adsorption of phosphate ions to form apatite. In our study, a Si-rich layer was formed on all Sr-CaSiO₃ ceramics after soaking in SBF and apatite layer was formed on the Si-rich layer formed, indicating that the mechanism of apatite formation was not altered. The ability to form apatite on CaSiO₃ ceramics has not been influenced by the incorporation of Sr, as shown in the results obtained on the intensity of apatite peaks in XRD and the thickness of apatite. A plausible explanation is that with an increase of the soaking time of the Sr-CaSiO₃ ceramics in SBF, the concentrations of Ca ions increase and can become supersaturated which is sufficient for apatite crystallization and growth. Interestingly, the morphology of the formed apatite on different Sr containing CaSiO₃ ceramics varied. This morphological difference can be attributed to the alteration of Ca ion concentrations

in SBF after soaking different Sr containing CaSiO₃ ceramics.

The ISO standard method was used to evaluate the effect of ions released from Sr-CaSiO₃ on HBDC proliferation. It was found that CaSiO₃ with no Sr promoted HBDC proliferation only at higher extract concentration and at low concentration the HBDC proliferation was comparable to the blank control. However, with the incorporation of Sr into CaSiO₃, the HBDC proliferation was stimulated even at low extract concentration. Sr was found to induce osteoblast activity [10–13] by stimulating bone formation and reducing bone resorption [14–16] by virtue of its great affinity to bone [17]. Previous studies indicated that Sr incorporation into porous calcium polyphosphate scaffolds and hydroxyapatite ceramics stimulated osteoblast proliferation [13,34]. Previous studies also showed that Ca and Si ions released from materials can stimulate osteoblast proliferation, differentiation and gene expression, which can be regarded as one of the evaluation criterion for bioactivity of the materials [35–37]. Our results showed that the high concentration of Ca and Si ions can promote HBDC proliferation and at the low concentration, Ca and Si have no stimulatory effect on HBDC proliferation (Table 1 and Fig. 10). However, with the incorporation of Si, even low concentration range of Ca and Si ions, promoted HBDC proliferation, suggesting that the incorporation of Sr into CaSiO₃ stimulated HBDC proliferation. Therefore, it is