Abstract: A calcium oxide-silica composite biomaterial either in amorphous state or crystalline state having an average pore size, as determined by the BET method, in the range of from 0.8 to 4 nm, wherein the calcium oxide-silica content of the biomaterial is at least 80 wt %, the balance being optionally one or more other materials and wherein the molar ratio of calcium oxide to silica is at least 0.1.
BIOMATERIALS, THEIR PREPARATION AND USE

FIELD OF THE INVENTION

The present invention relates to calcium oxide-silica composite biomaterials having a particular average pore size, to methods for their preparation and to uses thereof.

BACKGROUND

Apatite is a mineral that is produced and used by biological systems. The name apatite refers to a group of phosphate minerals, including hydroxyapatite, fluoroapatite and chloroapatite (having high concentrations of hydroxyl, fluoride and chloride anions respectively in the crystal lattice). The formula of the admixture of the three most common species of apatite is Ca₅(PO₄)₂Si(OH, F, Cl).

Hydroxyapatite is the major component of tooth enamel and a large component of bone material. It is a naturally occurring form of apatite, having the formula Ca₅(PO₄)₂OH (usually written as Ca₁₀(PO₄)₂Si(OH)₂, to denote that the crystal unit cell comprises two molecules).

Typically, hydroxyapatite has a prism-like shape with a width of about 60 nm and a length of several micrometers. The prism crystallites generally are aligned in a highly ordered manner.

It is known to use hydroxyapatite as a bone replacement material and as a coating on metal implants to promote bone in-growth, for example into prosthetic implants. Newly formed hydroxyapatite on implant surfaces helps to stimulate cells to secrete growth factors and to promote new tissue growth to form good bonding with implants. It is also known to use hydroxyapatite where remineralisation is required, for example in remineralisation of tooth enamel and bone, i.e. to treat osteoporosis. By the term "remineralisation", we mean restoring of depleted mineral content.
There has, therefore, been considerable interest in providing compositions for and methods of inducing hydroxylapatite formation.

Crystallised hydroxylapatite may be formed by sintering various calcium phosphate compounds at a given ratio and at a temperature above 600°C. The crystallised hydroxylapatite may then be ground into powder and blended with a polymer matrix, for use as a dental or bone implant. In use, the crystallised hydroxylapatite is dissolved in body fluid and induces the formation of new hydroxylapatite on or in a tooth or bone. However, this process is very slow and the formation of new hydroxylapatite takes a long time, often from several months to a year.

In an attempt to speed up the formation of hydroxylapatite a new type of cement system was developed. For example, US-4,612,053 describes a cement system that comprises tetracalcium phosphate (Ca₄(PO₄)₂O) and at least one other sparingly soluble calcium phosphate solid, such as dicalcium phosphate anhydrous (CaHPO₄). The cement forms hydroxylapatite when it is mixed with sodium phosphate solution. However, the cement produces a lot of heat upon hydroxylapatite formation, which harms surrounding tissue. Additionally, the cement is costly and difficult to make.

Porous biomaterials have also been developed in an attempt to enhance bioactivity and new bone in-growth.

Porous materials are classified into several kinds according to their size. For example, microporous materials have pore diameters of less than 2 nm, mesoporous materials have pore diameters between 2 and 50 nm and macroporous materials have pore diameters of greater than 50 nm.

An example of a type of porous biomaterial is a porous bioactive glass.

Bioactive glasses comprise SiO₂, CaO, P₂O₅, Na₂O and small amounts of other oxides. A bioactive glass typically has the basic formula CaO-P₂O₅-Na₂O-SiO₂. Bioactive glasses may be made by melt processes or by sol-gel processing (see, for example, Hench, J. Am. Ceram. Soc, 81, 7, 1705-28, (1998) and Hench, Biomaterials, 19 (1998), 1419-1423).
Bioactive glasses are known to bond to living bone. When a bioactive glass is immersed in body fluid, it is believed that calcium and phosphate ions migrate from the bioactive glass so as to form a calcium-phosphate rich surface layer. The layer below the calcium-phosphate rich surface becomes increasingly silica rich due to the loss of calcium ions. Upon exposure to water, silica forms Si-OH bonds. The hydroxyl group attracts calcium ions and the calcium ions attract phosphate ions so as to precipitate and transform into more stable hydroxylapatite (as suggested by Kokubo, "Apatite formation on surface of ceramics metals", Acta mater., Vol. 46, No. 7, 2519-2527, 1998). Thus, a layer of hydroxylapatite is formed on the bioactive glass. Cells then adhere to the layer of hydroxylapatite and gradually attach firmly to the bioactive glass so as to lay down an extracellular matrix. The matrix mineralises so as to connect with the bone tissue. Thus the bone bonds to the bioactive glass.

US-B-6,338,751 describes a bioactive glass composition including particulate bioactive and biocompatible glass containing 40 to 60% SiO₂, 10 to 30% CaO, 10 to 35% Na₂O, 2 to 8% P₂O₅, 0 to 25% CaF₂ and 0 to 10% B₂O₃ (where are percentages are by weight) and a particle size range less than 90 µm and including an effective dentin tubule occluding amount of particles less than about 10 µm.

US-A-2004/0087429 describes a bioactive glass comprising 30 to 60 mol% CaO, 40 to 70 mol% SiO₂ and 20 mol% or less Na₂O and its use in bone restoration materials.

WO-A-2005/063185 describes non-aqueous compositions comprising bioactive glass particles. The bioactive glass may comprise from 40 to 86% SiO₂, from 0 to 35% Na₂O, from 4 to 46% CaO and from 1 to 15% P₂O₅ (where are percentages are by weight).

CN-1554607 describes mesoporous and macroporous biological glass produced through surfactant self-assembling and sol-gel processes using surfactants and polymer beads. The surfactants used in the processes are E₀₂₀P₀₆₀E₀₂₀ (P123), E₀₁₀P₀₆₀E₀₁₀ (F127), E₀₁₃₂P₀₇₉E₀₁₃₂ (F108), E₀₂₀P₀₅₀E₀₂₀ (P65) and E₀₂₆P₀₉₀E₀₂₆ (P85), wherein EO is poly(ethylene)oxide and PO is poly(propylene)oxide. The polymer beads used in the processes are polystyrene and polybutyl methacrylate. The glasses produced include
phosphate ions. The glasses produced using the surfactants P123, P65 and P85 have average pore sizes of 4.6 nm, 5.1 nm and 6.0 nm respectively.

Hench et al. (Journal of Sol-Gel Science and Technology, 7, 59-68, 1996) describes gel-silica glasses having different pore sizes and teaches that the larger pore sizes are preferred.

Yu et al. (Angew. Chem. Int. Ed., 2004, 43, 5980-5984) describes a process for making highly ordered mesoporous bioactive glasses. The process comprises dissolving a nonionic block copolymer, tetraethyl orthosilicate (TEOS), calcium nitrate, triethyl phosphate and hydrochloric acid in ethanol and stirring the solution at room temperature to produce a sol. The sol then undergoes an evaporation-induced self-assembly (EISA) process and the dried gel is calcined at 700 °C to obtain the mesoporous bioactive glass. The nonionic block polymers are used as structure-directing agents to provide the desired pore size and structure. The nonionic block copolymers used are EO20PO70EO20 (P123), EO7OePO70EOePO70EO70 (F127) and EO39BO47EO39 (B50-6600), wherein EO is poly(ethylene)oxide, PO is poly(propylene)oxide and BO is poly(butylene)oxide. The mesoporous glasses formed by this process apparently are homogeneous and have a pore size in the range of from 4 to 7 nm. Yu et al. teaches that the mesoporous glasses formed by this process have superior bone-forming bioactivity in vitro.

It is known to use other surfactants as structure-directing agents for forming mesoporous and microporous materials.

For example, cetyltrimethylammonium bromide (CTAB) is known as a porogen molecule (or structure-directing agent) in mesoporous silica (see S. Mann et al., Adv. Mater. 2002, 14, No. 11, June 5, pages 1 to 14).

Stucky et al. (Science, Vol. 279, 1998, pages 548-552) describes the use of cationic cetyltrimethylammonium surfactants to make MCM-41 (a mesoporous silica having a hexagonal porous structure) having uniform pore sizes of from 2 to 3 nm. Stucky et al. also teaches that well-ordered hexagonal mesoporous silica structures with tunable large uniform pore sizes of up to 30 nm can be formed using amphiphilic block copolymers as organic structure-directing agents.
Holmberg et al. (Soft Matter., 2005, 1, 219-226) describes the use of cationic and nonionic surfactants as structure-directing agents to make mesoporous silica.

None of the aforementioned prior art documents disclose a calcium oxide-silica composite biomaterial that has an average pore size in the range of from 0.8 to 4 nm or a method for preparing such calcium oxide-silica composite biomaterials.

SUMMARY OF THE INVENTION

A first aspect of the present invention provides a calcium oxide-silica composite biomaterial either in amorphous state or crystalline state having an average pore size, as determined by the BET method, in the range of from 0.8 to 4 nm, wherein the calcium oxide-silica content of the biomaterial is at least 80 wt%, the balance being optionally one or more other materials and wherein the molar ratio of calcium oxide to silica is at least 0.1.

A second aspect of the present invention provides a method for preparing a calcium oxide-silica composite biomaterial either in amorphous state or crystalline state having an average pore size, as determined by the BET method, in the range of from 0.8 to 4 nm, wherein the calcium oxide-silica content of the biomaterial is at least 80 wt%, the balance being optionally one or more other materials and wherein the molar ratio of calcium oxide to silica is at least 0.1, the method comprising the steps of:

(i) combining, in solution, a calcium salt, an organic or inorganic silica precursor such as a silicate or a tetra(alkyl)silicate and a structure-directing agent in the presence of an aqueous solvent whereby hydrolysis of the tetra(alkyl)silicate occurs, leading to the formation of a sol;

(ii) isolating a solid from the sol; and

(iii) calcinating the isolated solid.

The calcium oxide-silica composite biomaterials of the present invention provide very real advantages in use. For example, they are easy to prepare and are capable of inducing the formation of hydroxyapatite over a much shorter period of time than the prior art biomaterials discussed above. Uses include tissue regeneration, tooth and/or bone regeneration, tooth whitening and treating and/or preventing tooth hypersensitivity.
Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art.

**Calcium Oxide-Silica Composite Biomaterials**

According to the present invention, there is provided a calcium oxide (Coo)-silica (SiO$_2$) composite biomaterial having an average pore size in the range of from 0.8 nm to 4 nm.

For the avoidance of any doubt, by the term "composite" we mean a single material formed of at least two different materials. The calcium oxide-silica composite biomaterials of the present invention comprise at least calcium oxide and silica. In other words, the calcium oxide-silica composite biomaterials comprise at least calcium, oxygen and silicon atoms bonded together to form the biomaterials.

As the skilled person would appreciate, the calcium oxide-silica composite biomaterials of the present invention may comprise additional components (i.e. in addition to calcium oxide and silica). Any such additional components may be included, provided that they do not inhibit or prevent the calcium oxide-silica composite biomaterials of the present invention from inducing the formation of hydroxyapatite as discussed in more detail below. In other words, it is preferred that any additional components do not participate in and/or inhibit the action of the calcium oxide-silica composite biomaterials in forming hydroxyapatite. This is because the present inventors believe that calcium oxide and silica alone are particularly effective in inducing the formation of hydroxyapatite in a solution containing phosphate ions, for example a body fluid or saliva. Any additional components may, for example, be included in an amount of less than 15% by weight, more preferably of less than 10% by weight.

For the avoidance of any doubt, by the term "biomaterial" we mean a material that is capable of bonding to human and/or animal tissue, including living tissue (such as bone tissue and tooth dentin) and non-living tissue (such as tooth enamel) and also including both soft and hard tissue.
The average pore size is that measured using the BET method. This may be performed using a commercially available instrument.

In another aspect of the present invention, the average pore size is in the range of from 2 to 4 nm, particularly in the range of from 2 to less than 4 nm, for example in the range of from 2 to 3.9 nm, particularly in the range of from 2 to 3.5 nm, more particularly in the range of from 2 to 3 nm.

In another aspect of the present invention, the average pore size is in the range of from 1 to 2.7 nm and in yet another aspect of the present invention, the average pore size is in the range of from 1.35 to 2.45 nm.

As the skilled person would appreciate, it is not essential for 100% of the pores to be of the specified pore size in order for the calcium oxide-silica composite biomaterials of the present invention to exhibit the advantages discussed above. The term "average pore size" is widely used in the art and would be understood by a person skilled in the art. The average pore size is calculated by a known statistical method.

The average pore size is controlled and selected by the use of an appropriate structure-directing agent during the formation of the calcium oxide-silica composite biomaterial, for example using the method described herein. In other words, the structure-directing agent is selected so as to provide the desired average pore size. As the skilled person would appreciate, the particular average pore size obtained depends on the particular structure-directing agent used. Other factors may also affect the average pore size, such as, for example, the pH and temperature of the preparation solution and the concentration of the structure-directing agent.

The calcium oxide-silica composite biomaterials of the present invention are in the amorphous state or crystalline state. Mixtures of biomaterials of both states are also within the ambit of the invention. Amorphous state includes the glass state.

The calcium oxide-silica composite biomaterials of the present invention typically are silica-based materials. In other words, the biomaterials comprise a primary structure of
silica, i.e. interconnected silicon and oxygen atoms. The particular structure formed by the network of interconnected silicon and oxygen atoms may be any suitable structure and will depend on several factors, including the nature of the structure-directing agent used to prepare the composite biomaterial. For example, when the structure-directing agent is CTAB typically a hexagonal porous structure is formed and when the structure-directing agent is F127® typically a cubic porous structure is formed. Calcium atoms are covalently bonded to the oxygen atoms in the silicon-oxygen network, so as to form a coherent and continuous mix of silicon, oxygen and calcium atoms. The composite material typically has a spherical shape once formed.

Typically, the pores of the calcium oxide-silica composite biomaterial have an ordered arrangement. The ordering of the pores can, for example, be detected by small angle X-ray diffraction, for example at angles of from 1 to 8° (compared to angles of 10 to 80° used for a normal crystal). Small angle X-ray diffraction is required because the pore size is larger than the atom crystal lattice. As the skilled person would appreciate, if the pores do not have an ordered arrangement, no peaks are observed in the small angle X-ray diffraction pattern. If, however, the pores have an ordered arrangement, a sharp peak is observed in the X-ray diffraction pattern.

The present inventors surprisingly have found that the calcium oxide-silica composite biomaterials of the present invention are especially effective at inducing the formation of hydroxylapatite, for example compared to the prior art biomaterials discussed above.

As discussed above, it is believed that in order for hydroxylapatite to form, calcium ions must be released from an appropriate biomaterial. In particular, in order for hydroxylapatite to form on the surface of a biomaterial, calcium ions must migrate to the surface of the material. Without wishing to be bound by any theory, it is believed that the average pore sizes of the calcium oxide-silica composite biomaterials of the present invention, which are small compared to many known biomaterials, provide a higher inner surface area, which allows for easy and efficient dissolution of the calcium atoms. Typically, the inner surface area of the calcium oxide-silica composite biomaterials of the present invention is in the range of from 400 to 1000 m²/g. Furthermore, the calcium oxide-silica composite biomaterials of the present invention include a well-ordered arrangement of pores and channels. Without wishing to be bound by any theory, it is believed that this well-
ordered arrangement allows easy transport of the calcium ions to the surface of the biomaterial, so as to aid the formation of hydroxylapatite. Additionally, it is believed that the small pore sizes prevent or reduce the formation of hydroxylapatite in the pores, so as to avoid blockage of the pore channel and thus increase the amount of hydroxylapatite that is formed at the surface, as desired.

The calcium oxide-silica composite biomaterials of the present invention may comprise calcium and silicon in any suitable ratio, provided that the molar ratio of calcium oxide to silica is at least 0.1. For example, the molar ratio of calcium to silicon may be in the range of from 1:10 to 1:1, for example in the range of from 1:10 to 1:2, particularly about 1:10. It is believed that this molar ratio helps to control the rate of release of calcium atoms from the composite biomaterial and, as the skilled person would appreciate, the optimum molar ratio will depend on the particular composite biomaterial and the conditions under which it is used.

Preferably, the calcium oxide-silica composite biomaterials of the present invention are substantially free of phosphate ions. By the term "substantially free" we mean that the composite biomaterials typically include less than 5% by weight, particularly less than 2.5% by weight, more particularly less than 1% by weight, even more particularly less than 0.5% by weight, of phosphate ions. For example, it is possible to prepare a calcium oxide-silica biomaterial of the present invention containing less than 0.005% by weight of phosphate ions using high purity starting materials, for example using calcium nitride supplied by China National Pharmaceutical Group Corporation (SINOPHARM), Beijing, China in a purity of greater than 99%.

The calcium oxide-silica composite biomaterials of the present invention that are substantially free of phosphate ions are believed to be advantageous because, in use, the formation (and precipitation) of calcium phosphate in the pores of the biomaterial is reduced. Instead, the calcium ions are able to migrate to the surface of the biomaterial before combining with the phosphate ions from aqueous solution to form calcium phosphate. This aids the formation of hydroxylapatite at the outer surface of the biomaterial. These biomaterials are in contrast to conventional biomaterials that included phosphate ions in their structure. Additionally, the calcium oxide-silica composite biomaterials of the present
invention that are substantially free of phosphate ions are believed to be advantageous because they have a simple composition and are easy to prepare.

The composite biomaterials of the present invention may contain one or more other materials provided, that the calcium oxide-silica content is at least 80 wt%. Although phosphate is a non-preferred such other material, the other materials(s) are typically selected from those commonly found in bioglasses.

As discussed above, in many applications it is preferred to form the hydroxylapatite at the surface of the biomaterial. This allows for the subsequent cascade of physiochemical interactions to occur that are required to form a bond to the tissue. Examples of such applications include coatings on metal implants, for example on titanium oxide implants.

Typically, the calcium oxide-silica composite biomaterials of the present invention are in the form of a powder. This is advantageous because it allows the materials to be used in powder form without requiring the step of forming a powder, for example by grinding into a powder form. Furthermore, the powder typically comprises particles of a small size that cannot readily be obtained by simple grinding processes, for example of a submicron size.

In one aspect of the invention, the calcium oxide-silica composite biomaterials of the present invention may be in the form of a glass. In order to form a glass, a suitable calcination temperature should be used, for example a calcination temperature of at least 900°C.

Method

According to the present invention, there is provided a method for preparing a calcium oxide-silica composite biomaterial having an average pore size in the range of from 0.8 to 4 nm, the method comprising the steps of:

(i) combining, in solution, a calcium salt, a tetra(alkyl)silicate and a structure-directing agent in the presence of an aqueous solvent whereby hydrolysis of the tetra(alkyl)silicate occurs, leading to the formation of a sol;
(ii) isolating a solid from the sol; and
(iii) calcininating the isolated solid.
With above described method it is also possible to produce calcium oxide-silica composite biomaterial having an average pore size in the range of from 2 to 4 nm, particularly in the range of from 2 to less than 4 nm, for example in the range of from 2 to 3.9 nm, particularly in the range of from 2 to 3.5 nm, more particularly in the range of from 2 to 3 nm.

With above described method it is also possible to produce calcium oxide-silica composite biomaterial having an average pore size of from 1 to 2.7 nm and in yet another aspect of the present invention, the average pore size is in the range of from 1.35 to 2.45 nm.

In step (i) of the method of the present invention, the tetra(alkyl) silicate (such as TEOS) is hydrolysed to form silica. As the skilled person would appreciate, it is not necessary for all of the tetra(alkyl) silicate to be hydrolysed. Typically at least 80% by weight of the tetra(alkyl) silicate is hydrolysed in step (i).

As the skilled person would appreciate, any suitable tetra(alkyl) silicate may be used in step (i) of the method of the present invention. Suitable tetra(alkyl) silicates include tetraethyl orthosilicate (hereinafter referred to as "TEOS") and tetramethyl orthosilicate. It is less preferred to use tetramethyl orthosilicate because tetramethyl orthosilicate produces methanol during the hydrolysis reaction. Methanol is known to be harmful to humans and animals. Also, methanol potentially may disrupt the formation of the ordered structure in the sol.

Any suitable concentration of tetra(alkyl) silicate may be used in step (i) of the method of the present invention. Suitable concentrations include 0.1 to 1M, particularly 0.3 to 0.6M.

As the skilled person would appreciate, any suitable calcium salt may be used in step (i) of the method of the present invention. Suitable calcium salts include those that are substantially soluble in an aqueous solution with a pH between 8 and 10. For example, suitable calcium salts include calcium nitrate, and calcium chloride. In one aspect, the calcium salt is calcium nitrate.
It is possible in step (i) of the method of the present invention, for some of the calcium salt to hydrolyse to form calcium hydroxide. This typically will only occur at pH values of greater than 8.

Any suitable concentration of calcium salt may be used in step (i) of the method of the present invention. The concentration is selected so as to provide the desired ratio of calcium and silicon, as discussed above.

As the skilled person would appreciate, any suitable structure-directing agent may be used in step (i) of the method of the present invention, provided that it is capable of forming a calcium oxide-silica composite biomaterial having an average pore size in the range specified. For example, the structure-directing agent may be a cationic or a nonionic surfactant and should be organic in nature. Suitable structure-directing agents are disclosed in Berggren et al., Soft Matter., 2005, 1, 219-226.

Suitable structure-directing agents include, for example, cationic surfactants of the general formula \( C_nH_{2n+1}N(CH_3)X \), wherein \( X \) represents bromo or chloro and \( n \) is 8, 10, 12, 14 or 16. When the structure-directing agent is such a surfactant, the calcium oxide-silica composite biomaterial produced typically has an average pore size in the range of from about 1.7 to 2.7 nm.

An example of such a cationic surfactant is cetyltrimethylammonium bromide (hereinafter referred to as “CTAB”), which has the formula \( \text{C}_{16}\text{H}_{33}\text{N(CH}_3\text{)}_3\text{Br} \) (i.e. \( n \) is 16). CTAB is commercially available (for example from Acros Organics, New Jersey, USA). When the structure-directing agent is CTAB, the calcium oxide-silica composite biomaterial produced has an average pore size of about 2.7 nm.

Further suitable structure-directing agents include, for example, nonionic surfactants of the general formula \( C_m\text{H}_{2m+2}+i\text{NH}_2 \), wherein \( m \) is 8, 10, 12, 14, 16 or 18. When the structure-directing agent is such a surfactant, the calcium oxide-silica composite biomaterial produced typically has an average pore size in the range of from about 1.6 to 2.4 nm.

An example of such a nonionic surfactant is dodecylamine, which has the formula \( \text{H}_2\text{N(Cl}_2\text{H}_2\text{)}_5 \) (i.e. \( m \) is 12). Dodecylamine is commercially available (for example from Tokyo
Kasei Kogyo Company Limited, Japan. When the structure-directing agent is dodecylamine, the calcium oxide-silica composite biomaterial produced has an average pore size of about 2.4 nm.

A further suitable structure-directing agent is Pluronic F88®, which is a nonionic block copolymer surfactant of the formula \(\text{EO}_{100}\text{PO}_{39}\text{EO}_{100}\), wherein EO represents poly(ethylene)oxide and PO represents poly(propylene)oxide. Pluronic F88® is commercially available (for example from BASF Corporation). When the structure-directing agent is Pluronic F88®, the calcium oxide-silica composite biomaterial produced has an average pore size of about 3.5 nm.

A further suitable structure-directing agent is Tetronic 908®, which is a nonionic star copolymer surfactant of the formula \((\text{EO}_{113}\text{PO}_{22})\text{N(CH2)2N(PO22EO}_{113})\text{2}\), wherein EO represents poly(ethylene)oxide and PO represents poly(propylene)oxide. Tetronic 908® has an average molecular weight of 25000. Tetronic 908® is commercially available (for example from BASF Corporation). When the structure-directing agent is Tetronic 908®, the calcium oxide-silica composite biomaterial produced has an average pore size of about 3.0 nm.

In one aspect of the present invention, the structure-directing agent is selected from cetyltrimethylammonium bromide (CTAB), Pluronic F88®, Tetronic 908® and dodecylamine, and mixtures thereof. In particular, the structure-directing agent may be CTAB.

Any suitable concentration of structure-directing agent may be used in step (i) of the method of the present invention and will depend on the particular structure-directing agent(s) being used. Suitable concentrations include, for example, 50 to 100 mM when the structure-directing agent is CTAB and 4 to 12 mM when the structure-directing agent is dodecylamine.

As the skilled person would appreciate, mixtures of one or more calcium salts, tetra(alkyl)silicates and/or structure-directing agents may be used in step (i) of the method of the present invention. It is, however, preferred to use only one structure-directing agent, as this aids the formation of pores of the desired size.
The aqueous solvent may comprise water and an alcohol. Any suitable alcohol may be used, for example a C<sub>1</sub>-C<sub>4</sub> alcohol such as methanol or ethanol (especially ethanol). The use of ethanol is advantageous because it is inexpensive and is not harmful to health during production.

The water in the aqueous solvent is required in order for the aforementioned hydrolysis reaction(s) to occur. Thus, the aqueous solvent must contain a sufficient amount of water to allow the hydrolysis reaction(s) to occur. Typically the aqueous solvent comprises between 40 and 50% by weight, for example about 45% by weight, of water. The amount of alcohol controls the rate of the hydrolysis reaction(s). As the amount of alcohol is increased, the rate of hydrolysis of the tetra(alkyl)silicate is decreased.

As the hydrolysis reaction(s) proceeds in step (i) of the method of the present invention, the silica and the calcium salt typically form a sol. The hydrolysed tetra(alkyl)silicate typically undergoes a condensation reaction, which leads to the formation of the desired network of interconnected silicon and oxygen atoms. For the avoidance of any doubt, by the term "sol", we mean a dispersion of colloidal particles in a liquid.

Without wishing to be bound by any theory, it is believed that above the critical micelle concentration, the structure-directing agent forms micelles, i.e. spherical or cylindrical structures that maintain the hydrophilic parts of the structure-directing agent in contact with water while shielding the hydrophobic parts within the micellar interior. The micelles then undergo a self-assembly process to form a three-dimensional structure within the sol. By the term "self-assembly" we mean the spontaneous organisation of the micelles through non-covalent interactions, such as hydrogen bonding, Van der Waals forces, electrostatic forces, \( \pi-\pi \) interactions (see, for example, Advanced Materials, Vol. 11, Issue 7, pages 579 to 585).

The particular three-dimensional structure formed by the micelles depends on several factors, including the interfacial tension energy of the micelles and the remainder of the solution. The interfacial tension energy is directly related to the interfacial area. The smallest interfacial energy is obtained by providing the smallest interfacial area, i.e. by closely packed micelles. The micelles closely pack when they are packed in an ordered
manner. Thus, the most ordered packing of the micelles provides the lowest energy state or the thermodynamically favoured state.

Without wishing to be bound by any theory, it is believed that the calcium and silica molecules are then attracted to the surfaces of the micelles by an electrostatic force. For example, the silica and calcium oxide materials assemble between and around ordered surfactant micelles, possibly due to the matching of charge density at the interfaces of the inorganic materials and the surfactants (see, for example, Kresge et al., Nature, Vol. 359, pages 701 to 712, 1992 and Huo et al., Nature, Vol. 368, pages 317 to 321, 1994).

The hydrolysis reaction of step (i) of the method of the present invention may be conducted at any suitable pH. The pH that is suitable will depend on the nature of the structure-directing agent used and will be selected so as to aid the formation of a micelle structure. For example, when the structure-directing agent is a cationic surfactant, step (i) typically is conducted at a basic pH (such as a pH in the range of from 8 to 10, particularly a pH of about 8). When the structure-directing agent is a nonionic surfactant, step (i) typically is conducted at an acidic pH (such as a pH of less than 2, particularly of less than 1).

Step (i) is conveniently carried out at a temperature in the range of, for example, from 20 to 40°C, conveniently at or near 25°C.

It is preferred in step (i) to first dissolve the calcium salt and the structure-directing agent in the aqueous solvent and to then add the tetra(alkyl)silicate to the solution.

If it is intended to prepare a biomaterial that contains phosphate, phosphate ions may be included in the reaction mixture in step (i). However, it is preferred that step (i) is conducted in the absence of phosphate ions, so as to produce a calcium oxide-silica composite biomaterial that is substantially free of phosphate ions. As discussed above, it is believed that this is advantageous because it minimises the formation (and precipitation) of calcium phosphate in the pores of the biomaterial, which in turn aids the formation of hydroxylapatite at the outer surface of the biomaterial.

As the skilled person would appreciate, the step (ii) of isolating a solid from the sol that is formed in step (i) may be conducted by any suitable method or means. For example,
the solid may be isolated by simply filtering off the aqueous solvent from the sol. Alternatively, the solid may be isolated by allowing the aqueous solvent to evaporate from the sol.

In step (ii), it is preferred to include the step of washing the isolated solid with water so as to remove any free ions from the solid before step (iii) is conducted. This is especially preferred when the solid is isolated by simply filtering off the aqueous solvent from the sol.

In step (iii) of the method of the present invention, the structure-directing agent is removed from the solid for example by thermal decomposition. In this way, the structure-directing agent is believed to direct the formation of a three-dimensional ordered pore architecture and to direct the formation of specifically sized pores.

It is believed that there is a linear relationship between the unit cell parameter (i.e. the pore size plus pore wall thickness) and the size of the structure-directing agent, for example the number of carbon atoms in the surfactant chain, in acidic and basic media respectively. Typically, the longer the carbon chain of the surfactant then the larger the pore size obtained.

Typically, in step (iii) the calcium salt is converted to calcium oxide (for example, when the calcium salt added in step (i) is calcium nitrate, it is converted to calcium oxide and nitrogen dioxide). The removal of the structure-directing agent provides an ordered three-dimensional pore structure.

As the skilled person would appreciate, the calcination step (iii) may be conducted at any suitable temperature. Suitable temperatures for the calcination step (iii) are those at which the structure-directing agent is thermally decomposed and the calcium salt is substantially converted to calcium oxide. Typically all of the calcium salt is converted to calcium oxide in the calcination step (iii).

As the skilled person would appreciate, the preferred calcination temperature varies according to the particular structure-directing agent used. Typically, the calcination step (iii) is conducted at a temperature in the range of, for example, from 500 to 800°C, particularly of from 500 to 700°C, even more particularly of from 500 to 600°C, for example about 550°C.
At these temperatures, the calcium oxide-silica composite biomaterial typically is formed as a powder. It does not form in a glass state or as a monolith (i.e. a single block of material). However, as the skilled person would appreciate, alternative calcination temperatures can be selected if it is desired to form a glass or a monolith.

According to the present invention, there is also provided a calcium oxide-silica composite biomaterial obtainable by a method as defined above. There is also provided a calcium oxide-silica composite biomaterial obtained by a method as defined above.

Uses

According to present invention, there is also provided the use of a calcium oxide-silica composite biomaterial as herein defined for inducing the formation of hydroxylapatite. The hydroxylapatite typically is formed on the surface of the calcium oxide-silica composite biomaterial.

In order to induce the formation of hydroxylapatite, it is believed that the calcium oxide-silica composite biomaterial of the present invention should be contacted with phosphate ions, for example with an aqueous solution containing phosphate ions at a minimum concentration of about 5 mM. Suitable solutions include, for example, phosphate buffer solution, artificial saliva, simulated body fluid and real human or animal saliva. Upon contact with the solution, the calcium oxide dissolves so as to release calcium ions into solution. The calcium ions and phosphate ions allow the system to reach hydroxylapatite supersaturation level (and hydroxylapatite formation) in a short period of time.

The composition of simulated body fluid in 1 litre solution is:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>8.03618</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>0.350</td>
</tr>
<tr>
<td>KCl</td>
<td>0.224</td>
</tr>
<tr>
<td>K₂HPO₄·3H₂O</td>
<td>0.2303</td>
</tr>
<tr>
<td>MgCl₂·6H₂O₂</td>
<td>0.31122</td>
</tr>
<tr>
<td>HCl (1 mol/L)</td>
<td>40 ml</td>
</tr>
</tbody>
</table>
CaCl₂ 0.383476
Na₂SO₄ 0.0717
(CH₂OH)₃CNH₂ 0.069138

In artificial saliva solution, the calcium ion concentration is 0.9 mM and the phosphate ion concentration is 7mM and the rest of the compositions are the same as simulated body fluid in the present study.

The calcium oxide-silica composite biomaterials of the present invention are believed to be advantageous because, in use, they induce hydroxylapatite formation by an efficient and fast mechanism. As discussed above, it is believed that the calcium oxide-silica composite biomaterials of the present invention effectively release calcium ions into solution and can control the crystallisation site. It is believed that the calcium oxide-silica composite biomaterials of the present invention direct the formation of hydroxylapatite at the surface of the biomaterials. In most cases of tissue regeneration, the formation of hydroxylapatite at the surface of the biomaterial is the prerequisite step for the subsequent reactions of that bond the tissue to the cells.

Typically, the calcium oxide-silica composite biomaterials of the present invention can induce the formation of hydroxylapatite in a faster time than the biomaterials of the prior art. For example, the biomaterials of the present invention typically can induce the formation of hydroxylapatite in a time period of less than 4 hours. After 24 hours, typically about 40% by weight of the calcium oxide-silica composite biomaterials will have been transformed into crystalline hydroxylapatite.

According to another aspect of the present invention, there is provided a method of forming hydroxylapatite, the method comprising the step of contacting the calcium oxide-silica composite biomaterial as herein defined with phosphate ions at a pH in the range of from 5 to 10, particularly of from 6.8 to 7.2, for example at a pH of about 7. The preferred pH depends on the particular application. For example, for the formation of hydroxylapatite in vivo, the preferred pH is in the range of from 6.8 to 7.2, for example about 7.

Typically, at least 98% by weight of the hydroxylapatite forms on the surface of the biomaterial. This can be confirmed by X-ray diffraction, scanning electron microscopy (SEM)
and transmission electron microscopy (TEM). The hydroxylapatite formed is preferably in a crystalline state.

The phosphate ions may, for example, be provided in solution, for example in a phosphate buffer solution, in artificial saliva, in simulated body fluid or in real human or animal saliva.

According to another aspect of the present invention, there is provided the use of a calcium oxide-silica composite biomaterial as herein defined in tissue regeneration. In this aspect, the tissue may be soft or hard tissue. By the term "regeneration", we include restoration and remineralisation processes.

According to another aspect of the present invention, there is provided the use of a calcium oxide-silica composite biomaterial as herein defined in tooth and/or bone regeneration. For example, we include the restoration of tooth dentin and bone, as well as the remineralisation of tooth dentin and tooth enamel. The remineralisation is intended to form new tooth or bone tissue, but not necessarily to restore the old tooth or bone to its original state. In the remineralisation process, hydroxylapatite is deposited onto the substrate (such as bone or tooth) and incorporated into the substrate at any location where there is a crack or lesion. Etching of the substrate surface before the hydroxylapatite is deposited may help to incorporate the hydroxylapatite internally into the substrate.

According to another aspect of the present invention, there is provided the use of a calcium oxide-silica composite biomaterial as herein defined for whitening a tooth.

According to another aspect of the present invention, there is provided the use of a calcium oxide-silica composite biomaterial as herein defined for treating and/or preventing tooth hypersensitivity.

The calcium oxide-silica composite biomaterials of the present invention may be used alone but will generally be administered in the form of a composition in which the calcium oxide-silica composite biomaterial is in association with an acceptable carrier. Typically, the composition will take the form of a paste, gel or cement. The composition may also take the form of a powder, which may be applied to a substrate on a strip (for example
Bondi strip) or as a spray (for example in combination with an inactive powder, such as silica or calcium carbonate powder).

Thus, according to another aspect of the present invention, there is provided a composition comprising a calcium oxide-silica composite biomaterial as herein defined.

According to another aspect of the present invention, there is provided a tissue regeneration composition comprising a calcium oxide-silica composite biomaterial as herein defined.

According to another aspect of the present invention, there is provided a bone regeneration composition comprising a calcium oxide-silica composite biomaterial as herein defined.

According to another aspect of the present invention, there is provided a tooth regeneration composition comprising a calcium oxide-silica composite biomaterial as herein defined.

According to another aspect of the present invention, there is provided a tooth whitening composition comprising a calcium oxide-silica composite biomaterial as herein defined.

According to yet another aspect of the present invention, there is provided a composition for treating and/or preventing tooth hypersensitivity, which composition comprises a calcium oxide-silica composite biomaterial as herein defined.

The compositions of the present invention may be in any suitable form, such as in the form of a cement, a paste or a gel. The compositions of the present invention may comprise any suitable carrier, such as a polymer gel carrier.

According to another aspect of the present invention, there is provided a toothpaste comprising a calcium oxide-silica composite biomaterial as herein defined. The toothpaste may comprise any suitable additional ingredients, such as ingredients selected from silica, calcium carbonate, surfactant, perfume and water, and mixtures thereof.
The amount of calcium oxide-silica composite biomaterial that is combined with the carrier(s) will necessarily vary depending upon nature of the material to which and the area to which it is to be applied and on the particular route of administration. A suitable ratio of calcium oxide-silica composite biomaterial to carrier is, for example, in the range of from 1:100 to 1:1.

The present invention further provides a method for the preparation of composition of the invention, which method comprises the step of combining a calcium oxide-silica composite biomaterial as herein defined with an acceptable carrier.

The present invention also provides the use of a calcium oxide-silica composite biomaterial as herein defined for inducing the formation of hydroxylcarbonate apatite. Typically, the hydroxylcarbonate apatite is formed on the surface of the calcium oxide-silica composite biomaterial.

In order to induce the formation of hydroxylcarbonate apatite, it is believed that the calcium oxide-silica composite biomaterial of the present invention should be contacted with phosphate ions in the presence of carbon dioxide. Suitable sources of phosphate ions are discussed above.

According to another aspect of the present invention, there is provided a method of forming hydroxylcarbonate apatite, the method comprising the step of contacting the calcium oxide-silica composite biomaterial as herein defined with phosphate ions at a pH in the range of from 5 to 10 (particularly of from 6.8 to 7.2, for example at a pH of about 7) in the presence of carbon dioxide. The formation of hydroxylcarbonate apatite is desirable in the regeneration of bone tissue.

The present invention will now be described further with reference to the following examples which are illustrative only and non-limiting.

In the examples, the average pore size of the biomaterials was measured by BET nitrogen sorption. The instrument used was a Micromeritics Tristar 3000 analyzer (from Micromeritics GmbH, Mönchengladbach, Germany). The nitrogen adsorption
measurements were performed at 77K in nitrogen gas using 0.1 g biomaterial as a powder. The powder was preheated at 200°C for 2 hours before testing to eliminate water.

The BET specific surface area was measured using a TriStar 3000 Analyzer, which uses physical adsorption and capillary condensation principles to obtain information about the surface area and porosity of a solid material. A sample contained in an evacuated sample tube was cooled to cryogenic temperature and then exposed to analysis gas at a series of precisely controlled pressures. With each incremental pressure increase, the number of gas molecules adsorbed on the surface increases. The equilibrated pressure (P) was compared to the saturation pressure (Po) and their relative pressure ratio (P/Po) was recorded along with the quantity of gas adsorbed by the sample at each equilibrated pressure. As adsorption proceeds, the thickness of the adsorbed film increases. Any micropores in the surface are filled first, then the free surface becomes completely covered, and finally the larger pores are filled by capillary condensation. The process may continue to the point of bulk condensation of the analysis gas. Then, the desorption process may begin in which pressure systematically is reduced resulting in liberation of the adsorbed molecules. As with the adsorption process, the changing quantity of gas on the solid surface at each decreasing equilibrium pressure is quantified. These two sets of data describe the adsorption and desorption isotherms. Analysis of the shape of the isotherms yields information about the surface and internal pore characteristics of the material.

The instrument used for the X-ray diffraction (XRD) measurements was a Rigaku, D/Max, 2500, Japan. This instrument utilizes the monochromatic X-rays to determine the interplanar-spacings (d-spacing) of the biomaterials. Samples were analysed as powders with grains in random orientations to insure that all crystallographic directions are "sampled" by the beam.

The instrument used for the scanning electronic microscopy (SEM) was a JEOL, JSM-6700F Field emission (made by JEOL, Japan).

The instrument used for the high resolution- transmission electronic microscopy (HRTEM) measurements was a JEOL, 2010F (made by JEOL, Japan). The 2010F is an energy filtering, field-emission analytic TEM/STEM. It operates at 200kV and uses a Schottky field emitter.
The instrument used for Raman spectroscopy measurements was a LabRam-1B, HORIBA Jobin Yvon Ltd, UK.

5 BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will now be explained in more detail by way of the following Example and with reference to the accompanying drawings in which:

Figure 1 shows pore size distributions for a material according to the present invention and a comparative material;

Figure 2 shows a small angle X-ray diffraction pattern for a material according to the present invention;

Figure 3 shows SEM images of calcined CaO-SiO$_2$ composite materials before and after incubation;

Figure 4 shows X-ray diffraction patterns for another material according to the present invention and of a comparative example;

Figure 5 shows SEM images of a calcined CaO-SiO$_2$ composite material according to the present invention and of a comparative example (scale bar 1 µm);

Figure 6 shows SEM images of the materials of Example 3;

Figure 7 shows SEM images of the materials of Example 4 (scale bar 1 µm);

Figure 8 shows SEM images of a tooth covered with a material according to the present invention, before and after incubation;

Figure 9 shows HRTEM images for the material of Example 1, after incubation;

Figure 10 shows a larger area HRTEM image of the sample depicted in Figure 9;

Figures 11 - 13 show HRTEM images obtained from the material of Comparative Example 1; and

Figures 14 shows a respective Raman spectra of an etched tooth sample before and after treatment with the material of Example 1.

30 EXAMPLES

Example 1
Cetyltrimethylammonium bromide (CTAB) powder (1.3 g) was dissolved in a mixture of deionised water (25 g) and ethanol (30 g). The weight ratio of CTAB to liquid is 0.02. The solution was stirred at 25°C for 10 minutes, after which time the CTAB had dissolved and the solution appeared clear. Ca(NO₃)₂ (2.36 g) was then added to the CTAB solution. Ammonia solution (25%, 1.6 g) was then added to obtain a pH of about 8. The solution was still transparent, which indicated that Ca(OH)₂ had not formed yet and calcium was in free ion form. Then liquid TEOS (6 g) was added drop-wise to the basic solution, with violent stirring. After 1 hour, the clear solution became cloudy. This showed that TEOS had started to hydrolyse. The stirring was continued for 24 hours at 25°C, until most of the solution had become a sol.

The hydrolysis product of the sol was then vacuum filtered and washed twice with deionised water (to remove the free ions in the solution). Then the solid collected by filtration was dried at 100°C for 12 hours. Finally the dried solid was calcinated at 550°C for 5 hours and allowed to cool in the oven to burn off the CTAB so as to form the pores. At this temperature, calcium nitride was also decomposed into CaO and NO₂. The SiO₂-CaO composite did not form a glass state and was not a monolith. Instead, the composite was a powder.

Figure 1 (a) shows the pore size distribution of the material measured by BET nitrogen sorption. The average pore size was found to be 2.7 nm. The measured BET specific surface area was 880.1 m²/g.

Figure 2 shows the small angle X-ray diffraction pattern of the material. A rising peak is shown at 2.5°, which is indicative of mesopore formation as well as the ordering of the pores.

The powder was then ground in a pestle and the ground powder (0.2 g) was poured into phosphate buffer solution (PBS, 30 ml) in a Pyrex glass bottle. PBS was prepared by dissolving Na₂HPO₄ (3.533 g) and KH₂PO₄ (3.387 g) in deionised water (1 litre) at a pH of 6.8. Three different concentrations of PBS were used to evaluate the effect of the phosphate concentration. These were (1) normal PBS prepared with a phosphate concentration of 24.9 mM, (2) 5 times dilute, called PBS-Dilute 5, and (3) 10 times dilute, called PBS- Dilute 10. The mouth of the glass bottle was sealed. Then plastic was wrapped
around the glass mouth and the sealed bottle (containing the powder sample and the PBS solution) was placed in a water bath incubator (Model: DKZ-Z; Company name: Shanghai Fuma Experimental Equipment Co. Ltd., Shanghai, China) with a gentle shaking at a temperature of 37°C (± 0.1 °C). Samples (2 to 3 ml) were removed after 1 hour, 4 hours, 8 hours, 1 day and 12 days. These samples were quickly transferred into a refrigerator for freezing to keep them in their original state until they were characterised by X-ray diffraction, SEM and TEM.

Figure 3 shows SEM images of the calcinated CaO-SiO₂ composite material before (Figure 3 (a)) and after 1 days incubation (Figure 3 (b)). The samples showed that full crystalline hydroxylapatite had formed after 1 day.

Comparative Example 1

The procedure of Example 1 was repeated using Pluronic F127® instead of CTAB. Pluronic F127® (6 g) was added to deionised water (30 g) with stirring at 60°C. Then HCl (2M, 112 g) was added to the solution. Liquid TEOS (12 g) was added drop-wise to the acidic solution and the resulting solution was stirred vigorously for 24 hours. The solution became cloudy after 12 hours.

Figure 1 (b) shows the pore size distribution of the material measured by BET nitrogen sorption. The average pore size was found to be 4.9 nm. The measured BET specific surface area was 400.1 m²/g.

Example 2

The calcinated CaO-SiO₂ composite material from Example 1 (0.1 g) was incubated in a phosphate buffer solution (PBS, 25mM, 30 ml) at a pH of 6.8. The incubation temperature was set to 37°C. After incubation for 1 day, the sample was taken out, filtered and dried.

The dried sample was characterised by X-ray diffraction (XRD). The XRD spectrum of this sample is shown in Figures 4 (a) and (b), both before and after incubation for 1 day. Clearly, before incubation, there are no sharp peaks showing that only the amorphous phase
existed (Figure 4 (a)). After incubating for 1 day, a full pattern of XRD peaks appeared, which peaks were confirmed as representing hydroxylapatite, by using software produced by Materials Data, Inc (which software can identify phases in a sample, characterize density and lattice constants) indicated as triangle symbols (Figure 4 (b)). All of the peaks shown relate to hydroxylapatite, showing that the hydroxylapatite was the only product of the incubated sample. No other impurity or other types of calcium phosphate were present. The mature pattern of the hydroxylapatite XRD peaks also provided direct information for the fully-grown out new phase of hydroxylapatite.

The microscopic morphology of the samples was observed before and after they were incubated, using scanning electronic microscopy. Figure 5 shows SEM images of the calcinated CaO-SiO₂ composite material from Example 1 before (Figure 5 (a)) and after 1 day incubation (Figure 5 (b)). Before incubation, the material was in a spherical shape and had a smooth surface. The spherical diameter of the particles was between 0.2 and 0.5 µm and most of them were aggregated. After incubating in a PBS solution at 37°C for 1 day, full crystalline hydroxylapatite was grown out from the spherical particle substrate. The hydroxylapatite crystals were in a plate-like shape and of a size of between 1 to 10 µm (i.e. much larger than its substrate particle). These platelet hydroxylapatite crystals were covering almost all the substrate surface of calcinated CaO-SiO₂ powder, forming a full but not dense layer of hydroxylapatite.

Comparative Example 2

Example 2 was repeated using the calcinated CaO-SiO₂ composite material from Comparative Example 1.

The dried sample was characterised by X-ray diffraction (XRD). The XRD spectrum of this sample is shown in Figures 4 (c) and (d), both before and after incubation for 7 days. Clearly, before incubation, there were no sharp peaks showing that only the amorphous phase existed (Figure 4 (c)). After incubating for 7 days, a weak pattern of XRD peaks corresponding to hydroxylapatite appeared (Figure 4 (d)).

The microscopic morphology of the calcinated CaO-SiO₂ composite material from Comparative Example 1 was observed before and after they were incubated, using scanning
electronic microscopy (SEM). Figure 5 shows SEM images of the calcinated CaO-SiO₂ composite material before (Figure 5 (c)) and after 7 days incubation (Figure 5 (d)). Before incubation, the calcinated CaO-SiO₂ material from Comparative Example 1 was irregular and had a smooth surface. After incubating these particles into PBS solution at 37°C for 7 days, star-like hydroxylapatite crystallites were grown out of the original smooth substrate, as shown in Figure 5 (d). The average size of the hydroxylapatite crystal plates was less than 0.5 μm, much smaller than that from the calcinated CaO-SiO₂ composite material from Example 1.

Discussion of Example 2 and Comparative Example 2

A comparison of Example 2 and Comparative Example 2 shows that the calcinated CaO-SiO₂ material of Example 1 produces hydroxylapatite in a more mature crystalline form (because the hydroxylapatite crystallite produced are much bigger in size) and in larger quantities than the calcinated CaO-SiO₂ composite material of Comparative Example 1. Surprisingly, the time it took to form hydroxylapatite was much shorter for the calcinated CaO-SiO₂ composite material of Example 1 than for the calcinated CaO-SiO₂ composite material of Comparative Example 1. The calcinated CaO-SiO₂ composite materials of Example 1 and of Comparative Example 1 have substantially the same chemical composition and differ only in their pore size, as discussed above.

In summary, XRD and SEM results have demonstrated that calcinated CaO-SiO₂ composite material from Example 1 has much higher capability to induce hydroxylapatite formation than the calcinated CaO-SiO₂ composite material from Comparative Example 1. The former can produce more hydroxylapatite crystallites in shorter incubation time.

Example 3

A much shorter incubation time was tested for the sample of the calcinated CaO-SiO₂ composite material from Example 1. The procedure of Example 2 was repeated, except that samples were removed after incubation for 1 hour, 4 hours and 8 hours. Then scanning electronic microscopy (SEM) was used to observe the morphology change. The results are shown in Figure 6, in which (a) is the sample before incubation and (b), (c), (d) are the samples after incubation for 1 hour, 4 hours and 8 hours respectively. Before incubation, the
sample had a smooth surface and a spherical shape (see Figure 6 (a)). After incubation for 1 hour, fully crystalline hydroxylapatite had grown with a platelet-like shape (see Figure 6 (b)). After incubation for 4 hours, the HA plates had grown larger, gradually covering all of the substrate (see Figure 6 (c)). After incubation for 8 hours, the HA plates had formed a full rounded pattern (see Figure 6 (d)).

Example 4

The procedure of Example 3 was repeated, except that a control sample was included. The control sample was prepared in the same way as the calcinated CaO-SiO₂ composite material from Example 1 except that no structure-directing agent (i.e. no CTAB) was included. Figures 7 (a) to (c) show SEM images for the calcinated CaO-SiO₂ composite material from Example 1 after 1, 4 and 8 hour incubation times. Figure 7 (d) shows the SEM image for the control sample after incubation for 24 hours. Even after 1 hour incubation time, the calcinated CaO-SiO₂ composite material from Example 1 formed fully crystallized hydroxylapatite plates (Figure 7 (a)). The plates were relatively small (around 1 µm in its edge dimension) and grew out into a blossom-like pattern, starting from one location and forming a large cluster of greater than 5 µm in its lateral dimension. After 4 hours incubation, the calcinated CaO-SiO₂ composite material from Example 1 produced more hydroxylapatite flower-like clusters in higher density (Figure 7 (b)). After 8 hours incubation time, the calcinated CaO-SiO₂ composite material from Example 1 produced a fully-fledged, spherical, peony-flower like hydroxylapatite crystal, of about 10 µm in diameter (Figure 7 (c)). In comparison, the SEM image for the control sample after incubation for 24 hours shows that not a single crystal plate formed (Figure 7 (d)). Very smooth spherical particles remained and no new phase had formed.

Example 5

The calcinated CaO-SiO₂ composite material from Example 1 was applied in the form of a gel to treat a damaged human tooth.
The gel was prepared by mixing the calcinated CaO-SiO$_2$ composite material from Example 1 (0.2 g) with a carrier material (0.5 g) and a phosphate buffer solution (pH 7, 50 mM) at a temperature in the range of from 60 to 80°C with quick stirring. The solution was then cooled to room temperature and a white gel formed.

The gel was then applied to a human tooth and incubated for 1 day at 37°C in a simulated oral fluid (having a calcium concentration of 0.9 mM and a phosphate concentration of 7 mM). The gel-coated tooth was then washed three times with distilled water and observed using SEM. Figure 8 shows the SEM results. Before incubation, there are many micro size cracks on the surface of the tooth enamel (see Figure 8 (a)). After incubation with the gel, a uniform covering layer of hydroxylapatite was coated on the cracked tooth surface (see Figure 8 (b)).

Example 6

The nucleation behaviour of hydroxylapatite formed from the calcinated CaO-SiO$_2$ composite materials from Example 1 and Comparative Example 1 was studied by high resolution- transmission electronic microscopy (HRTEM).

Figure 9 shows HRTEM images for the calcinated CaO-SiO$_2$ composite material from Example 1 after incubation for 1 hour as described in Example 2. An embryonic crystalline hydroxylapatite starts to grow in a plane (112) from the edge of amorphous calcinated CaO-SiO$_2$ composite material from Example 1 (see Figure 9). The hydroxylapatite embryo is very small, marked by a white rectangle. Figure 10 shows a larger area view of the new hydroxylapatite phase formed from the calcinated CaO-SiO$_2$ composite material from Example 1. More needle-like hydroxylapatite crystallites are grown out from the edge of the spherical shape substrate. Those needles will further grow into a larger plate-like shape, for example as shown in Figure 7. During all the time that the calcinated CaO-SiO$_2$ composite material from Example 1 was observed by HRTEM, no nucleated hydroxylapatite crystallite was found inside the matrix of the material. This shows that the pores in this material suppress the nucleation. Without wishing to be bound by any theory, it is believed that this is due to size of the pores.
In comparison, the calcinated CaO-SiO$_2$ composite material from Comparative Example 1 was also observed by HRTEM following incubation for 7 days, as shown in Figures 11 to 13. Nanosize hydroxylapatite crystals were found to start the nucleation within the amorphous calcinated CaO-SiO$_2$ composite material from Comparative Example 1 (see Figure 11). The lattice constants were measured as 8.160 angstrom in the upper domain, representing hydroxylapatite (100) plane and 3.525 angstrom in the lower domain, representing hydroxylapatite (201) plane. The diameter of the newly formed hydroxylapatite domain is about 5 nm (comparable to the BET measured average diameter of 4.9 nm in calcinated CaO-SiO$_2$ composite material from Comparative Example 1). So, the hydroxylapatite is believed to seed its nuclei inside the pores and then the nuclei grow until they are of a size that fills the pores/channels between adjacent pores, indicated by the small regions/domains of hydroxylapatite. Figure 12 further shows that a well crystalline hydroxylapatite cluster was formed away from the surface of the parental matrix in a size of about 5 nm. However, another much larger hydroxylapatite crystallite was formed close to the surface of amorphous matrix, with its diameter larger than 5 nm. It is believed that this may be because the crystallite is located closer to the edge of the calcinated CaO-SiO$_2$ composite material from Comparative Example 1 and so it is not constrained by the pore size. A larger area view of crystalline hydroxylapatite from calcinated CaO-SiO$_2$ composite material from Comparative Example 1 is shown in Figure 13. Both needle and plate-like hydroxylapatite crystals are shown adjacent to the amorphous area. This shows that the hydroxylapatite is able to nucleate inside the pores of the calcinated CaO-SiO$_2$ composite material from Comparative Example 1.

**Example 7**

A human tooth was etched with 37wt% phosphate acid *in vitro*. The tooth was immersed in the acid solution for 60 seconds and then rinsed thoroughly to wash off the residue of phosphate acid.

The tooth was treated with a gel comprising the calcinated CaO-SiO$_2$ composite material from Example 1 (0.15 g), water (10 g) and gel carrier (0.15 g) and then incubated for one week at 37°C in a phosphate buffer solution of concentration of 50 mM.
Figures 14 (a) and (b) show SEM images of the tooth sample. Figure 14 (a) shows the tooth after the acid etching and Figure 14 (b) shows the acid etched tooth after treatment with the gel. It is clear from Figure 14 (b) that a thin layer of new repair coating was formed on the acid etched tooth sample.

Raman spectroscopy was used to detect the surface chemistry. Figure 14 (c) shows the Raman spectrum for the acid etched tooth without gel treatment and Figure 14 (d) shows the Raman spectrum for the tooth after treatment with the gel. The Raman spectra shown in Figures 14 (c) and (d) are identical, which means that they have identical surface chemical composition. This shows that the repairing layer formed by the gel is hydroxylapatite, the same material as the tooth enamel.
1. A calcium oxide-silica composite biomaterial either in amorphous state or crystalline state having an average pore size, as determined by the BET method, in the range of from 0.8 to 4 nm, preferably from 2 to 4 nm, more preferably from 2 to 3.5 nm, especially preferably from 2 to 3 nm.

2. A calcium oxide-silica composite biomaterial according to claim 1, wherein the average pore size is in the range of from 1 to 2.7 nm, preferably from 1.35 to 2.45 nm.

3. A calcium oxide-silica composite biomaterial according to claim 1 or 2 that is substantially free of phosphate ions.

4. A calcium oxide-silica composite biomaterial according to any of the preceding claim that is in the form of a powder.

5. A method for preparing a calcium oxide-silica composite biomaterial either in amorphous state or crystalline state having an average pore size, as determined by the BET method, in the range of from 0.8 to 4 nm, wherein the calcium oxide-silica content of the biomaterial is at least 80 wt%, the balance being optionally one or more other materials and wherein the molar ratio of calcium oxide to silica is at least 0.1, the method comprising the steps of:

(i) combining, in solution, a calcium salt, an organic or inorganic silica precursor such as a silicate or a tetra(alkyl)silicate and a structure-directing agent in the presence of an aqueous solvent whereby hydrolysis of the tetra(alkyl)silicate occurs, leading to the formation of a sol;

(ii) isolating a solid from the sol; and

(iii) calcinating the isolated solid.

6. A method according to claim 5, wherein the tetra(alkyl)silicate is selected from tetramethyl orthosilicate and tetraethyl orthosilicate.

7. A method according to claim 6, wherein the silica precursor is an organic precursor which is a tetra(alkyl)silicate in the form of tetraethyl orthosilicate.
8. A method according to any one or more of claims 5 to 7, wherein the calcium salt is selected from calcium nitrate, calcium fluoride and calcium chloride.

9. A method according to any one or more of claims 5 to 8, wherein the structure-directing agent is selected from a surfactant of the formula \( C_n \text{H}_{2n+1} \text{N(CH3)}_3 \text{X} \), wherein \( X \) is bromo or chloro and \( n \) is 8, 10, 12, 14 or 16, a surfactant of the formula \( C_m \text{H}_{2m+1} \text{NH}_2 \), wherein \( m \) is 8, 10, 12, 14, 16 or 18, Pluronic F88® and Tetronic 908®, and mixtures thereof.

10. A method according to claim 9, wherein the structure-directing agent is selected from cetyltrimethylammonium bromide, Pluronic F88®, Tetronic 908®, and dodecylamine.

11. A method according to any one or more of claims 5 to 10, wherein the aqueous solvent comprises water and a \( C_1-C_4 \) alcohol.

12. A method according to any one or more of claims 5 to 11, wherein in step (ii) the solid is isolated by filtration.

13. A method according to any one or more of claims 5 to 11, wherein in step (ii) the solid is isolated by evaporation.

14. A method according to any one or more of claims 5 to 13, wherein the calcination in step (iii) is conducted at a temperature in the range of from 500 to 800°C.

15. A calcium oxide-silica composite biomaterial obtainable by a method according to any one or more of claims 5 to 14.

16. The use of a calcium oxide-silica composite biomaterial according to any one or more of claims 1 to 4 and 15 for inducing the formation of hydroxyapatite.

17. A method of forming hydroxyapatite, the method comprising the step of contacting the calcium oxide-silica composite biomaterial according to any one or more of claims 1 to 4 and 15 with phosphate ions at a pH in the range of from 5 to 10.
18. The use of a calcium oxide-silica composite biomaterial according to any one or more of claims 1 to 4 and 15 in tissue regeneration.

19. The use of a calcium oxide-silica composite biomaterial according to any one or more of claims 1 to 4 and 15 in tooth and/or bone regeneration.

20. The use of a calcium oxide-silica composite biomaterial according to any one or more of claims 1 to 4 and 15 for whitening a tooth.

21. The use of a calcium oxide-silica composite biomaterial according to any one or more of claims 1 to 4 and 15 for treating and/or preventing tooth hypersensitivity.

22. A tissue regeneration composition comprising a calcium oxide-silica composite biomaterial according to any one or more of claims 1 to 4 and 15.

23. A bone regeneration composition comprising a calcium oxide-silica composite biomaterial according to any one or more of claims 1 to 4 and 15.

24. A tooth regeneration composition comprising a calcium oxide-silica composite biomaterial according to any one or more of claims 1 to 4 and 15.

25. A tooth whitening composition comprising a calcium oxide-silica composite biomaterial according to any one or more of claims 1 to 4 and 15.

26. A composition for treating and/or preventing tooth hypersensitivity, which composition comprises a calcium oxide-silica composite biomaterial according to any one or more of claims 1 to 4 and 15.

27. A calcium oxide-silica composite biomaterial generally as herein described with reference to the illustrative example(s).
Fig. 8(a).

Fig. 8(b).
Fig.9.
Fig. 10.
Fig. 11.
Fig. 12.
Fig. 13.
Fig. 14.

Before Treatment

Active powder in gel treated for 1