[Table 8]

Case				
Age	Sex	Embedding site	Length of fixture (mm)	Evaluation
45	male	maxilla molar	13	good
		maxilla molar	13*	normal
41	male	maxilla molar	13	good
		maxilla molar	11*	normal
45	male	maxilla molar	11	good
		maxilla molar	11*	normal
56	male	maxilla molar	13	good
		maxilla molar	13*	normal
40	male	maxilla molar	13	good
		maxilla molar	13*	normal
68	male	maxilla molar	11	good
		maxilla molar	13*	normal
50	male	maxilla molar	13	good
		maxilla molar	11*	normal
57	male	maxilla molar	13	good
		maxilla molar	13*	normal

*: untreated implant AstraTech Implant

[0158]

As shown Fig. 8, in the titanium implant of which surface was coated with the growth factor obtained from immortalized dens deciduous stem cell, progressed unification of the implant with the bone was shown in spite of the length of the fixtures.

[0159]

(Example 7) Analysis of the protein in the culture supernatant and immobilized on the surface of the sophisticated implant

(1) Material and Method

The proteins in the culture supernatant of the stem cell obtained from example 1 were used to be analyzed with LC/MS/MS (MS4 HPLC System (Michrom BioResources Inc.) and LCQ Advantage mass spectrometry system

collagen α-2(I) chain	collagen α-2(I) chain *	P02452
vimentin	-	P08670
collagen α-1(IV) chain	-	P12109
IGF-binding protein 7	IGF-binding protein 7	Q16270
fibronectin	fibronectin*	P02751
decorin	decorin*	P07585
plasminogen activator inhibitor 1	plasminogen activator inhibitor 1	P05121
actin, cytoplasmic 2	actin, cytoplasmic 2	P60709
sulfhydryl oxidase 1	sulfhydryl oxidase 1	O00391
SPARC	-	P09486
metalloproteinase inhibitor 1	-	P01033
collagen α-1(IV) chain	-	P08123
SCP2	-	Q9UJQ7
72 kDa type IV collagenase	-	P08253
B-2 microglobulin	-	AAA51811
Rho GTPase activate protein 18	-	Q8N392
-	follistatin relating protein 1	Q12841

[0163]

As shown in Table 9, it was clarified that a lot of protein relating to form cell structure was contained in the culture supernatant of the stem cell obtained in the example 1. Also, since the ration of the adherent cells obtained after culturing the mesenchymal stem cell obtained as mentioned above was high, it was thought that collagen α-1(I) chain, collagen α-2(I) chain, fibronectin and decorin played important roles in the adhesion of the implant and the alveolar bone.

[0164]

(Example 8) Influences by the culture supernatant to cell adherent property and by the lyophilization

(1) Material

The culture supernatant of stem cell obtained in the example 1 was used as the test sample and PBS as the negative control.

(2) Influence to the cell adherent property

Canine bone marrow stroma cells (10 mL of bone marrow fluid was

[Table 8]

Case				
Age	Sex	Embedding site	Length of fixture (mm)	Evaluation
45	male	maxilla molar	13	good
		maxilla molar	13*	normal
41	male	maxilla molar	13	good
		maxilla molar	11*	normal
45	male	maxilla molar	11	good
		maxilla molar	11*	normal
56	male	maxilla molar	13	good
		maxilla molar	13*	normal
40	male	maxilla molar	13	good
		maxilla molar	13*	normal
68	male	maxilla molar	11	good
		maxilla molar	13*	normal
50	male	maxilla molar	13	good
		maxilla molar	11*	normal
57	male	maxilla molar	13	good
		maxilla molar	13*	normal

*: untreated implant AstraTech Implant

[0158]

As shown Fig. 8, in the titanium implant of which surface was coated with the growth factor obtained from immortalized dens deciduous stem cell, progressed unification of the implant with the bone was shown in spite of the length of the fixtures.

[0159]

(Example 7) Analysis of the protein in the culture supernatant and immobilized on the surface of the sophisticated implant

(1) Material and Method

The proteins in the culture supernatant of the stem cell obtained from example 3 were used to be analyzed with LC/MS/MS (MS4 HPLC System (Michrom BioResources Inc.) and LCQ Advantage mass spectrometry system

collagen α-2(I) chain	collagen α-2(I) chain *	P02452
vimentin	-	P08670
collagen α-1(IV) chain	-	P12109
IGF-binding protein 7	IGF-binding protein 7	Q16270
fibronectin	fibronectin*	P02751
decorin	decorin*	P07585
plasminogen activator inhibitor 1	plasminogen activator inhibitor 1	P05121
actin, cytoplasmic 2	actin, cytoplasmic 2	P60709
sulfhydryl oxidase 1	sulfhydryl oxidase 1	O00391
SPARC	-	P09486
metalloproteinase inhibitor 1	-	P01033
collagen α-1(IV) chain	-	P08123
SCP2	-	Q9UJQ7
72 kDa type IV collagenase	-	P08253
B-2 microglobulin	-	AAA51811
Rho GTPase activate protein 18	-	Q8N392
-	follistatin relating protein 1	Q12841

[0163]

As shown in Table 9, it was clarified that a lot of protein relating to form cell structure was contained in the culture supernatant of the stem cell obtained in the example 3. Also, since the ration of the adherent cells obtained after culturing the mesenchymal stem cell obtained as mentioned above was high, it was thought that collagen α-1(I) chain, collagen α-2(I) chain, fibronectin and decorin played important roles in the adhesion of the implant and the alveolar bone.

[0164]

(Example 8) Influences by the culture supernatant to cell adherent property and by the lyophilization

(1) Material

The culture supernatant of stem cell obtained in the example 1 was used as the test sample and PBS as the negative control.

(2) Influence to the cell adherent property

Canine bone marrow stroma cells (10 mL of bone marrow fluid was

CLAIMS

1. A production method for sophisticated implant comprising the steps of:

washing a surface of an implant material with an organic solvent to prepare a washed implant material;

immersing said washed implant material in an first calcification solution having prescribed composition at prescribed temperature for 3 to 6 hours to prepare an immersed implant material; and

incubating said immersed implant material in a second calcification solution containing a cell product obtained from a bone marrow stromal cell or immortalized dens deciduous stem cell at a prescribed concentration for 2 to 5 days.

2. The preparation method for cell product according to the claim 1, wherein said bone marrow stromal cell is derived from rat femur, and said immortalized dens deciduous stem cell is derived from either swine dental pulp or human dens deciduous.

3. The cell product according to the claim 2, wherein said cell product comprises at least type I collagen (Col-I), fibronectin, decorin and vascular endothelial growth factor (VEGF).

4. The cell product according to the claim 3, wherein said cell product further comprises insulin-like growth factor-binding protein 7, follistatin related protein, metalloproteinase inhibitor 1, tissue plasminogen activator inhibitor, actin, and sulfhydryl oxidase I.

5. The cell product according to the claim 4, wherein said cell product further comprises SPARC, collagen α2 (IV) chain, β-2-microglobulin and Rho GTPase-activating protein 18.

6. The production method for the sophisticated implant according to the claim 1, wherein said implant material is formed by any material selected from the group consisting of titanium, zirconia, hydroxyapatite and β-TCP.

7. The production method for sophisticated implant according to the claim 1, wherein said organic solvent is acetone and 60 to 80% of ethanol.

8. The production method for the sophisticated implant according to the claim 1, wherein said first calcification solution is composed of 100 to 150 mM of NaCl, 2.5 to 3.5 mM of $CaCl_2 \cdot H_2O$, 1.5 to 2.5 mM of K_2HPO_4 and 25 to 75 mM of Tris-HCl (pH 7.2 to 7.6), and said second calcification solution is composed of 130 to 160 mM of KNO_3, 1 to 2 mM of $Ca(NO_3)_2 \cdot 4H_2O$ and 0.5 to 1.5 mM of K_2HPO_4 (pH 7.4).

9. The production method for the sophisticated implant according to the claim 8, wherein said first calcification solution is composed of 136.8 mM of NaCl, 3.10 mM of $CaCl_2 \cdot H_2O$, 1.86 mM of K_2HPO_4, and 50 mM of Tris-HCl (pH 7.4), and said second calcification solution is composed of 142.8 mM of KNO_3, 1.5 mM of $Ca(NO_3)_2 \cdot 4H_2O$, and 0.9 mM of K_2HPO_4 (pH 7.4).

10. The production method for the sophisticated implant according to the claim 1, wherein said prescribed temperature is from 36 to 38 °C and immersing time is 4 hours.

11. The production method for the sophisticated implant according to the claim 1, wherein said cell product of described concentration is at a concentration of 5 to 20 mg/mL.

12. A sophisticated implant produced by the method according to any one of the claims 1 to 11.

13. The sophisticated implant according to the claim 12, wherein the implant has at least type I collage, fibronectin and decorin on the surface.

14. The sophisticated implant according to the claim 13, wherein the implant further comprises at least one of protein selected from the group consisting of insulin-like growth factor - binding protein 7, follistatin related protein, metalloproteinase inhibitor I, tissue plasminogen activator inhibitor, actin and sulfhydryl oxidase I on the surface of the implant.

15. The sophisticated implant according to the claim 13, wherein said sophisticated implant is formed by a material selected from the group consisting of titanium, zirconia, hydroxyapatite and β-TCP.

CLAIMS

1. A production method for sophisticated implant comprising the steps of:

washing a surface of an implant material with an organic solvent to prepare a washed implant material;

immersing said washed implant material in an first calcification solution having prescribed composition at prescribed temperature for 3 to 6 hours to prepare an immersed implant material; and

incubating said immersed implant material in a second calcification solution containing a cell product obtained from a bone marrow stromal cell or immortalized dens deciduous stem cell at a prescribed concentration for 2 to 5 days.

2. The production method for sophisticated implant according to the claim 1, wherein said bone marrow stromal cell is derived from rat femur, and said immortalized dens deciduous stem cell is derived from either swine dental pulp or human dens deciduous.

3. The production method for sophisticated implant according to the claim 1, wherein said cell product comprises at least type I collagen (Col-I), fibronectin, decorin and vascular endothelial growth factor (VEGF).

4. The production method for sophisticated implant according to the claim 3, wherein said cell product further comprises insulin-like growth factor-binding protein 7, follistatin related protein, metalloproteinase inhibitor I, tissue plasminogen activator inhibitor, actin, and sulfhydryl oxidase I.

5. The production method for sophisticated implant according to the claim 4, wherein said cell product further comprises SPARC, collagen α2 (IV) chain, β-2-microglobulin and Rho GTPase-activating protein 18.

6. The production method for the sophisticated implant according to the claim 1, wherein said implant material is formed by any material selected from the group consisting of titanium, zirconia, hydroxyapatite and β-TCP.

7. The production method for sophisticated implant according to the claim 1, wherein said organic solvent is acetone and 60 to 80% of ethanol.

8. The production method for the sophisticated implant according to the claim 1, wherein said first calcification solution is composed of 100 to 150 mM of NaCl, 2.5 to 3.5 mM of $CaCl_2 \cdot H_2O$, 1.5 to 2.5 mM of K_2HPO_4 and 25 to 75 mM of Tris-HCl (pH 7.2 to 7.6), and said second calcification solution is composed of 130 to 160 mM of KNO_3, 1 to 2 mM of $Ca(NO_3)_2 \cdot 4H_2O$ and 0.5 to 1.5 mM of K_2HPO_4 (pH 7.0 to 7.8).

9. The production method for the sophisticated implant according to the claim 8, wherein said first calcification solution is composed of 136.8 mM of NaCl, 3.10 mM of $CaCl_2 \cdot H_2O$, 1.86 mM of K_2HPO_4, and 50 mM of Tris-HCl (pH 7.4), and said second calcification solution is composed of 142.8 mM of KNO_3, 1.5 mM of $Ca(NO_3)_2 \cdot 4H_2O$, and 0.9 mM of K_2HPO_4 (pH 7.4).

10. The production method for the sophisticated implant according to the claim 1, wherein said prescribed temperature is from 36 to 38 °C and immersing time is 4 hours.

11. The production method for the sophisticated implant according to the claim 1, wherein said cell product of described concentration is at a concentration of 5 to 20 mg/mL.

12. A sophisticated implant produced by the method according to any one of the claims 1 to 11.

13. The sophisticated implant according to the claim 12, wherein the implant has at least type I collage, fibronectin and decorin on the surface.

14. The sophisticated implant according to the claim 13, wherein the implant further comprises at least one of protein selected from the group consisting of insulin-like growth factor - binding protein 7, follistatin related protein, metalloproteinase inhibitor I, tissue plasminogen activator inhibitor, actin and sulfhydryl oxidase I on the surface of the implant.

15. The sophisticated implant according to the claim 13, wherein said sophisticated implant is formed by a material selected from the group consisting of titanium, zirconia, hydroxyapatite and β-TCP.