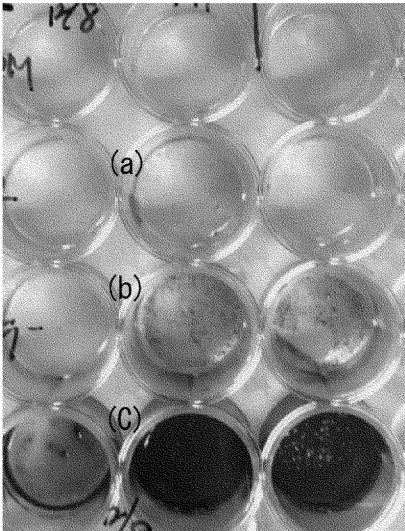


A Alizarin Red staining



B Light absorbance (OD 450 nm)

0.010	0.012	0.012
0.016	(a) 0.009	0.015
0.036	(b) 0.121	0.207
0.333	(c) 2.213	2.218

FIG. 1

C Alizarin Red staining (×40)

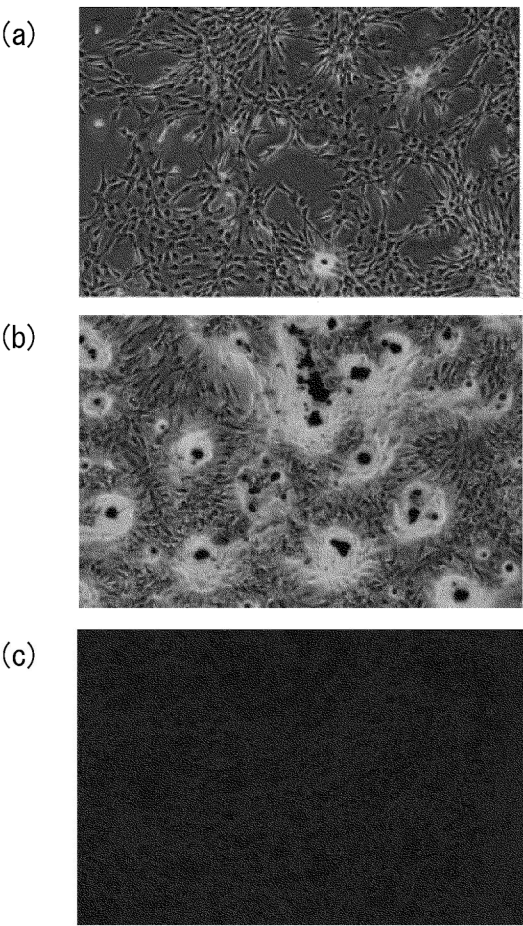
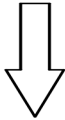


FIG. 2

(1) Switched to osteoblast differentiation-inducing culture medium at 80% to 90% confluence and cultured for 2, 4, 6 days



Detached after 2 days culture and reseeded in following culture media

(A) IMDM
(B) CM+IMDM
(C) CPPs+IMDM
(D) Osteoblast differentiation

} Culture →

Extracted and light absorbance measured after staining with Alizarin Red

After 5 days	After 8 days
0.023	0.014
0.039	0.028
0.051	0.078
0.688	1.943

Detached after 4 days culture and reseeded in following culture media

(A) IMDM
(B) CM+IMDM
(C) CPPs+IMDM
(D) Osteoblast differentiation

} Culture →

After 4 days	After 8 days
0.035	0.007
0.025	0.002
0.035	0.039
1.035	2.388

Detached after 6 days culture and reseeded in following culture media

(A) IMDM
(B) CM+IMDM
(C) CPPs+IMDM
(D) Osteoblast differentiation

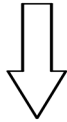
} Culture →

After 1 days	After 3 days	After 8 days
0.043	0.043	0.034
0.045	0.059	0.022
0.074	0.079	0.133
0.092	0.416	3.325

Osteoblast differentiation:
Osteoblast differentiation-inducing culture medium (positive control)

FIG. 3

(2) Switched to IMDM at 80% to 90% confluence, switched to osteoblast differentiation-inducing culture medium 2 days later at 100% confluence, and cultured for 2, 4, 6 days



Detached after 2 days culture and reseeded in following culture media

(A) IMDM
(B) CM+IMDM
(C) CPPs+IMDM
(D) Osteoblast differentiation

} Culture →

Extracted and light absorbance measured after staining with Alizarin Red

After 1 days	After 4 days	After 8 days
0.011	0.019	0.010
0.022	0.022	0.015
0.024	0.050	0.024
0.155	1.861	2.399

Detached after 4 days culture and reseeded in following culture media

(A) IMDM
(B) CM+IMDM
(C) CPPs+IMDM
(D) Osteoblast differentiation

} Culture →

After 3 days	After 8 days
0.068	0.024
0.049	0.027
0.060	0.058
0.314	3.088

Detached after 6 days culture and reseeded in following culture media

(A) IMDM
(B) CM+IMDM
(C) CPPs+IMDM
(D) Osteoblast differentiation

} Culture →

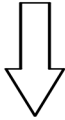
After 1 days	After 3 days	After 8 days
0.030	0.051	0.025
0.047	0.032	0.045
0.031	0.039	0.079
0.098	0.275	2.876

Osteoblast differentiation:

Osteoblast differentiation-inducing culture medium (positive control)

FIG. 4

(3) Switched to osteoblast differentiation-inducing culture medium at 100% confluence, and cultured for 2, 4, 6 days



Detached after 2 days culture and reseeded in following culture media

(A) IMDM
(B) CM+IMDM
(C) CPPs+IMDM
(D) Osteoblast differentiation

} Culture →

Extracted and light absorbance measured after staining with Alizarin Red

After 4 days	After 8 days
0.019	0.013
0.023	0.027
0.038	0.126
1.247	2.818

Detached after 4 days culture and reseeded in following culture media

(A) IMDM
(B) CM+IMDM
(C) CPPs+IMDM
(D) Osteoblast differentiation

} Culture →

After 1 days	After 4 days	After 8 days
0.021	0.024	0.031
0.019	0.026	0.033
0.028	0.039	0.245
0.168	0.892	2.478

Detached after 6 days culture and reseeded in following culture media

(A) IMDM
(B) CM+IMDM
(C) CPPs+IMDM
(D) Osteoblast differentiation

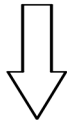
} Culture →

After 3 days	After 8 days
0.023	0.009
0.036	0.018
0.110	0.370
0.201	2.030

Osteoblast differentiation:
Osteoblast differentiation-inducing culture medium (positive control)

FIG. 5

(4) 2 days hyperproliferation from 100% confluence and then switched to osteoblast differentiation-inducing culture medium and cultured for 2, 4, 6 days



Detached after 2 days culture and reseeded in following culture media

(A) IMDM
(B) CM+IMDM
(C) CPPs+IMDM
(D) Osteoblast differentiation

} Culture →

Extracted and light absorbance measured after staining with Alizarin Red

After 4 days	After 8 days
0.028	0.025
0.016	0.008
0.034	0.055
1.260	2.566

Detached after 4 days culture and reseeded in following culture media

(A) IMDM
(B) CM+IMDM
(C) CPPs+IMDM
(D) Osteoblast differentiation

} Culture →

After 3 days	After 6 days	After 8 days
0.010	0.012	0.012
0.016	0.009	0.015
0.036	0.121	0.207
0.333	2.213	2.218

Detached after 6 days culture and reseeded in following culture media

(A) IMDM
(B) CM+IMDM
(C) CPPs+IMDM
(D) Osteoblast differentiation

} Culture →

After 1 days	After 3 days	After 8 days
0.015	0.020	0.011
0.016	0.041	0.365
0.243	0.347	1.748
0.091	0.778	2.628

Osteoblast differentiation:

Osteoblast differentiation-inducing culture medium (positive control)

FIG. 6

Seeded and cultured in plate after 2, 4, 6 days suspension at rest, and then after staining with Alizarin Red, pigment extracted, and light absorbance of pigment measured

Suspension at rest

Differentiated for 2 days in following culture media

(A) IMDM
(B) CM+ IMDM
(C) CPPs+ IMDM
(D) Osteoblast differentiation



Seeded in plate and cultured with each culture medium

(A) IMDM		After 4 days	After 8 days	(B) CM+ IMDM		After 4 days	After 8 days	(C) CPPs+ IMDM		After 4 days	After 8 days	(D) Osteoblast differentiation		After 4 days	After 8 days
0.016	0.021			0.064	0.032	0.022	0.194	0.022	0.234	0.022	0.158	1.014	1.828		
0.015	0.007			0.040	0.031	0.021	0.234	0.021	0.234	0.021	0.234	2.247	2.846		
0.018	0.005			0.047	0.029	0.027	0.146	0.027	0.146	0.027	0.146	1.161	1.705		
0.009	0.005			0.043	0.022	0.027	0.158	0.027	0.158	0.027	0.158	0.825	0.880		

Seeded in plate and cultured with each culture medium

(A) IMDM		After 4 days	After 8 days	(B) CM+ IMDM		After 4 days	After 8 days	(C) CPPs+ IMDM		After 4 days	After 8 days	(D) Osteoblast differentiation		After 4 days	After 8 days
0.019	0.012			0.038	0.036	0.068	0.283	0.068	0.283	0.068	0.283	1.011	1.142		
0.061	0.057			0.062	0.003	0.066	0.289	0.066	0.289	0.066	0.289	1.352	1.584		
0.018	0.034			0.041	0.060	0.071	0.143	0.071	0.143	0.071	0.143	1.287	0.877		
0.069	0.011			0.032	0.026	0.050	0.219	0.050	0.219	0.050	0.219	1.158	1.560		

Differentiated for 4 days in following culture media

(A) IMDM
(B) CM+ IMDM
(C) CPPs+ IMDM
(D) Osteoblast differentiation



Seeded in plate and cultured with each culture medium

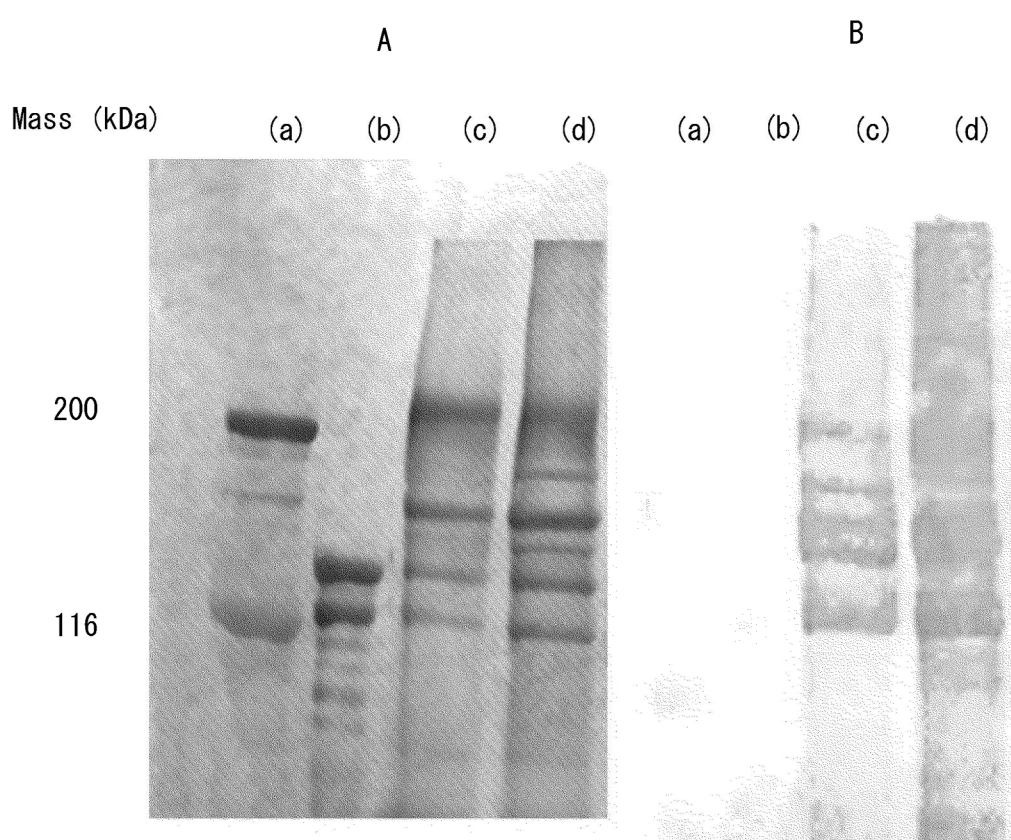
(A) IMDM		After 4 days	After 8 days	(B) CM+ IMDM		After 4 days	After 8 days	(C) CPPs+ IMDM		After 4 days	After 8 days	(D) Osteoblast differentiation		After 4 days	After 8 days
0.033	0.013			0.042	0.032	0.060	0.086	0.060	0.086	0.060	0.086	1.437	0.935		
0.022	0.012			0.039	0.025	0.064	0.067	0.064	0.067	0.064	0.067	1.113	1.048		
0.014	0.005			0.044	0.021	0.050	0.074	0.050	0.074	0.050	0.074	1.055	1.214		
0.017	0.004			0.028	0.023	0.059	0.073	0.059	0.073	0.059	0.073	1.526	1.145		

Differentiated for 6 days in following culture media

(A) IMDM
(B) CM+ IMDM
(C) CPPs+ IMDM
(D) Osteoblast differentiation



Osteoblast differentiation: Osteoblast differentiation-inducing culture medium (positive control)

FIG. 7

- (a) Marker
- (b) Type I atelocollagen
- (c) CPPs composition 1
- (d) CPPs composition 2