Study of corrosion of combinations of titanium/Ti-6Al-4V implants and dental alloys

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Metal ions released in 1% lactic acid solution from combinations of titanium fixtures with superstructures made of dental precious metal alloys (dental alloys) and titanium and differences based on the fixing method were investigated. In combinations of titanium with dental alloys, the level of Ti release was influenced by micro-structure of titanium: it was lower when the grain size was smaller. In titanium-titanium combinations, differences in the micro-structure of metal also markedly influenced the dissolution: the level of release increased when the micro-structure of titanium was different. The Ti and V release levels were higher in combination with titanium alloy and titanium than with titanium alloy and dental alloys. Regarding the superstructure-fixture fixing method, the level of Ti release was significantly lower in cement than in direct fixation.

Keywords: Implant, Dissolution of metal ions, Micro-structure of titanium

INTRODUCTION

Fixture-implanting treatment has recently been increasingly applied, and cement fixation is frequently employed to bind fixtures with superstructures because of its superiority to screw fixation regarding the esthetic appearance, fit accuracy, and procedure¹⁻⁵⁾. Because titanium and dental gold alloys are not in direct contact due to the cement, which it is an insulator, cement fixation of superstructures to fixtures does not produce a galvanic current and is less likely to cause corrosion. However, there has been no study on metal ion release due to differences in the fixation method.

Dental implants are comprised of several structures depending on the procedure and cases, such as fixtures, abutments, and fixation screws, and not only titanium but also titanium alloy is used for the composition. In addition, micro-structure of metal of some fixtures varies depending on the diameter⁶⁾, but its influence on metal ion release has not been investigated.

The important point on the *in vitro* evaluation of the corrosion resistance of dental metals is reproduction of the oral environment, for which selection of the test solution is significant. Solutions simulating the biological conditions include 0.9% NaCl solution, phosphate-buffered saline (PBS (-)), and artificial saliva⁷⁻¹⁰⁾. Yoneyama *et al.*¹¹⁾ pointed out that 0.9% NaCl solution reproduced the body fluid to some extent, but lacked quantitative properties, particularly, reliability of the released Ti ion level, and 1.0% lactic acid solution was suitable. Okazaki *et al.*¹²⁾ placed titanium alloy implants in the rat tibia, and observed that the metal level in tissue near the implant placement site was higher than the levels in calf serum and released in PBS (-) but lower than that released in

1.0% lactic acid solution, based on which they recommended 1.0% lactic acid solution as a suitable as well as simple solution for accelerated immersion testing. Accordingly, I adopted 1.0% lactic acid solution. On the other hand, natural electrode potentials generated between different metals, the potentiodynamic polarization curve, and changes in the galvanic current potential and density were reported in detail as electrochemical properties with regard to the evaluation of the corrosion resistance of biological metal materials¹³⁻¹⁸⁾. However, it is difficult to electrochemically reproduce clinically important conditions in the presence of influences of luting agents and adhesives used for fixation and between-space corrosion and the tightening stress of screw fixation.

In this study, I performed immersion tests of 5 types of combination of superstructures prepared with dental precious metal alloys (dental alloys), titanium, titanium alloy, and acrylic with titanium, titanium alloy, and acrylic as control fixtures in lactic acid solution, in order to investigate the following 4 items: 1) influences of fixation methods to bind fixtures with superstructures (direct and cement fixations); 2) influences of titanium fixture/superstructure combinations; 3) influences of the micro-structure of titanium fixture; and 4) influences of combination with the titanium alloy fixture. The levels of metal ion release and discoloration after immersion were measured, and the micro-structure of metal and surface were observed. These were also compared between the combinations bound by cement and direct fixations.

MATERIALS AND METHODS

Preparation of test samples

Experiments were performed using metal materials (3 types of titanium with different degrees of processing,

titanium alloy, 5 types of porcelain-fused-to-metal (PFM) alloy, 5 types of gold alloy, silver-palladium-copper-gold alloy, and silver alloy, presented with codes below) shown in Table 1, and acryl as a control, and 4 types of superstructure: those prepared with dental alloys cast by the lost wax method, lathed titanium and titanium alloy, and acryl. The diameter and length of superstructures were 6 and 4.8 mm, respectively. Fixtures were cut out from wires with a diameter and length of 4 and 17 mm, respectively, and the tip was 6-degree tapered over a 3.8-mm length (Fig. 1). The titanium superstructure, STi, and titanium fixture, FTi, were prepared with wire of 6- and 4-mm in diameter, Ti1 and Ti2, respectively (Table 1). The titanium superstructure, STi2, and titanium fixture, FTi2, were prepared with wire of 6-mm in diameter (Ti3) (Table 1). The titanium alloy (Ti-6Al-4V) superstructure, S64, and titanium alloy (Ti-6Al-4V) fixture, F64, were prepared with wire of 8.5-mm in diameter, Ti64 (Table 1). These were lathed using oil, and their surface roughnesses (R_a : μm) were FTi: 1.03, FTi2: 0.95, F64: 0.96, STi: 0.54, STi2: 0.57, and S64: 0.58. Dental alloys were cast into investment molds using a vacuum pressure casting machine (CASPAC 8000, Dentrone Co., Tokyo, Japan) according to manufacturer's instructions. Casted dental alloys were finally polished with Deerskin Buff (MOMOSE DENTAL MFG. Co., Osaka, Japan) and a polishing material, Multi Blue (DAIEI DENTAL PRODUCT Co., Osaka, Japan). Acryl used for the comparative control acrylic fixtures (FAC) was cut from wire of 4-mm in diameter, and that used for the acrylic superstructure

(SAC) was prepared by mechanically combining a pipe with outer and inner diameters of 6 and 4 mm, respectively, and 4-mm wire. Ti was ultrasonicated in acetone and distilled water, dental alloys in ethanol and distilled water, and acryl in distilled water. Superstructures and fixtures were bound employing 2 fixing methods: direct and cement fixations, and 5 combinations of the superstructure-fixture combinations

Fig. 1 Schematic diagram of test sample (superstructure and fixture).

Table 1 Metal materials used in experiments and their codes

Materials	Code* ¹	Trade name	Manufacturer	Composition (mass%)* ²
Titanium	Ti1	TITANIUM WIRE	Suzuki Metal Industry	99.839Ti-0.001H-0.10-0.01N-0.05Fe
	Ti2	TITANIUM WIRE	Nabekura Metal Industry	99.8342Ti-0.0028H-0.090-0.003N-0.06Fe-0.01C
	Ti3	TITANIUM WIRE	Nabekura Metal Industry	99.8343Ti-0.0017H-0.090-0.004N-0.06Fe-0.01C
Titanium Alloy	Ti64	TITANIUM ALLOY WIRE	Daido Steel	89.477Ti-6.21Al-4V-0.003H-0.150-0.01N-0.14Fe-0.01C
PFM Alloy* ³	C98	ZEO METAL 87	Yamamoto Precious Metal	87Au-11Pt-2(Zn, Ir)
	C94	SUPER CRYSTAL KP-5	Yamamoto Precious Metal	75Au-6.7Pt-12.3Pd-1.8Ag-4.2(In, Sn, Re, Ir, Ga, Fe, Cu)
	C83	QUINTESS CERAFY	Yamamoto Precious Metal	56Au-2Pt-24.5Pd-13Ag-4.5(In, Sn, Ru)
	C82	ZEO METAL 53	Yamamoto Precious Metal	53Au-1.5Pt-27.5Pd-12.3Ag-5.7(Sn, In, Ir, Cu, Ga)
	C61	ZEO METAL ST	Yamamoto Precious Metal	60.5Pd-27Ag-5.7Sn-5.4In-1.4(Zn, Ga, Ru)
Gold Alloy	G77	BENEFIT G	Yamamoto Precious Metal	70Au-4.5Pt-2Pd-13.6Ag-8.8Cu-1.1(Zn, Ir)
	G76	YP GOLD TYPE IV	Yamamoto Precious Metal	71Au-2Pt-3Pd-8Ag-15Cu-(Zn, Ir)
	G75A	B.I YELLOW	Yamamoto Precious Metal	71Au-4Pt-12.3Ag-12.1Cu-0.6(Zn, Ir)
	G75B	BENEFIT JUSTY	Yamamoto Precious Metal	68Au-7Pt-16.2Ag-8Cu-0.8(Zn, Ir)
	G58	SPACY J	Yamamoto Precious Metal	50Au-4.5Pt-3.2Pd-32.7Ag-9Cu-(Zn, Ir)
Ag-Pd-Cu-Au Alloy	GP	PALLA Z 12	Yamamoto Precious Metal	12Au-20Pd-50Ag-16.5Cu-1.5(Zn, In, Ga, Ir)
Silver Alloy	SL	UNICOM-7	Yamamoto Precious Metal	0.7Pd-70Ag-20.5In-6.2Zn-2.6(Sn, Ir, Al)

*¹: Code numbers of dental alloys indicate the total amount of precious metals.

*²: Composition is taken from inspection certificates and packaging labels.

*³: Porcelain-fused-to-metal alloy

Table 2 Materials used for fixtures and superstructure and fixation methods

	DB-FTi	CB-FTi	CB-FAc	DB-FTi2	DB-F64
Fixture	FTi	FTi	FAc	FTi2	F64
	STi,	STi,	STi,	STi2,	S64, STi,
Superstructure	Dental alloys (12 species)	Dental alloys (12 species),	Dental alloys (12 species)	Dental alloys (4 species),	Dental alloys (4 species)
		SAc		SAc	
Fixed method	Direct	Cement	Cement	Direct	Direct

with these 2 fixing methods were tested (Table 2).

FTi and superstructures prepared with 13 metals (STi, C98, C94, C83, C82, C61, G77, G76, G75A, G75B, G58, GP, and SL) bound by direct fixation were designated as DB-FTi and those by cement fixation as CB-FTi. As controls not combined with titanium, FAc and superstructures bound by cement fixation were designated as CB-FAc. FTi2 and superstructures prepared with 5 metals (STi2, C94, G76, GP, and SL) bound by direct fixation were designated as DB-FTi2, and the titanium alloy fixture F64 and superstructures prepared with 6 metals (S64, C94, G76, GP, SL, and STi) bound by direct fixation as DB-F64. Direct fixation was applied on the assumption of screw fixation, in which a 30-kg weight was loaded for 5 minutes for fitting using a constant-load tester (Nihon Mecu Co., Tokyo, Japan). In cement fixation, fixtures and superstructures were bound using adhesive glass ionomer resin cement (GC Fuji Luting S, GC Co., Tokyo, Japan) followed by loading a 5-kg weight using a constant-load tester. Excess cement was removed during the 5-minute loading. The test samples bound by cement fixation were kept in 37°C distilled water for 24 hours after weight loading. Seven test samples were prepared for each condition.

Immersion test

The test sample was immersed in 50 mL of 1% lactic acid solution and shaken 50 times/min for 3 months in a 37°C shaking incubator (IK400W, Yamato Scientific Co., Tokyo, Japan). The samples bound by direct fixation were immersed immediately after binding and those bound by cement fixation were immersed after keeping them in 37°C distilled water for 24 hours.

Quantification of metal ion release

After immersion, the test samples were taken out, metal ions that had been released into 1% lactic acid solution were measured 3 times using an Inductively Coupled Plasma-Atomic Emission Spectrometry (ICPE-9000, Shimadzu Co., Kyoto, Japan), and the mean was calculated employing the 3-point calibration method. In addition, the levels of Ti and V ion release per unit area were also determined. Metal ion measurement was limited to metals contained in the samples.

Measurement of color differences between before and after immersion

To measure color differences between before and after immersion, the reflectance at 300-800-nm wavelengths including regular reflection was measured at the occlusal surface of the superstructure 20 times before and after immersion in each test sample using a spectrophotometer (PMA-10, Hamamatsu Photonics K. K., Shizuoka, Japan), and the color difference (ΔE^*ab) was calculated from the means.

Observation of micro-structure of metal

Titanium and titanium alloy (STi, STi2, S64, FTi, FTi2, and F64) were embedded with acryl resin, polished to a mirror state using an automatic polisher (AUTOMETO 2/ECOMET 3, BUEHLER, Illinois, USA), and etched using chemical polishing solution for titanium (CHEMI-POLISH, SHOFU, Kyoto, Japan), followed by observation under a scanning confocal laser microscope (LEXT OLS3000, Olympus Co., Tokyo, Japan).

Surface observation

The test sample surfaces were observed under the scanning confocal laser microscope before and after immersion.

Statistical analysis

Bartlett's test was employed for tests classified by a single factor. One-way ANOVA was used when the distribution was homogenous, and the Kruskal-Wallis test was employed when the distribution was not homogenous. For tests classified by 2 factors, the Levene test was employed, two-way ANOVA was used when the distribution was homogenous, and Bonferroni's multiple comparison was applied when the distribution was not homogenous.

RESULTS

Quantification of metal ion release

Table 3 shows metal ion release from DB-FTi, CB-FTi, and CB-FAc. Seven ion metals: Ag, Cu, Ga, In, Sn, Zn, and Ti, were released. The highest total level of metal ion release was noted in the combinations with SL regardless of the condition, followed by gold alloys and GP, whereas releases from combinations with PFM alloys and titanium were low-level. Of trace elements contained in PFM alloys, non-precious metals, such as

Table 3 Total levels of metal ion release from DB-FTi, CB-FTi, and CB-FAc

Sample		Released ion concentration (ppm)							
Super-structure	Group	Ag	Cu	Ga	In	Sn	Ti	Zn	Total
STI	DB-FTi	-	-	-	-	-	1.419 (0.599)	-	1.419
	CB-FTi	-	-	-	-	-	0.1683 (0.034)	-	0.168
	CB-FAc	-	-	-	-	-	0.1156 (0.016)	-	0.116
C98	DB-FTi	-	-	-	-	-	0.246 (0.068)	11.495 (4.701)	11.741
	CB-FTi	-	-	-	-	-	0.1046 (0.046)	11.079 (10.320)	11.183
	CB-FAc	-	-	-	-	-	0.0203 (0.002)	3.062 (1.750)	3.082
C94	DB-FTi	-	-	0.125 (0.065)	1.111 (0.183)	0.058 (0.013)	0.254 (0.081)	-	1.548
	CB-FTi	Traces	-	0.295 (0.132)	1.293 (0.142)	0.080 (0.006)	0.0759 (0.007)	-	1.744
	CB-FAc	Traces	-	0.122 (0.044)	0.544 (0.103)	Traces	0.0160 (0.002)	-	0.682
C83	DB-FTi	Traces	-	-	0.710 (0.270)	0.066 (0.024)	0.235 (0.081)	-	1.011
	CB-FTi	Traces	-	-	0.304 (0.061)	Traces	0.0581 (0.022)	-	0.362
	CB-FAc	Traces	-	-	0.132 (0.043)	-	0.0216 (0.005)	-	0.154
C82	DB-FTi	Traces	-	0.124 (0.016)	0.849 (0.262)	0.060 (0.022)	0.272 (0.060)	-	1.305
	CB-FTi	Traces	-	0.048 (0.012)	0.467 (0.067)	Traces	0.0747 (0.010)	-	0.590
	CB-FAc	Traces	-	0.043 (0.011)	0.216 (0.035)	-	0.0173 (0.003)	-	0.276
C61	DB-FTi	0.019 (0.007)	-	0.083 (0.053)	0.309 (0.084)	Traces	0.186 (0.062)	-	0.597
	CB-FTi	0.256 (0.396)	-	0.071 (0.069)	0.437 (0.279)	0.058 (0.042)	0.0756 (0.011)	Traces	0.897
	CB-FAc	0.031 (0.004)	-	0.036 (0.008)	0.196 (0.037)	Traces	0.0182 (0.004)	-	0.281
G77	DB-FTi	0.013 (0.005)	21.157 (6.135)	-	-	-	0.240 (0.084)	5.236 (1.066)	26.646
	CB-FTi	Traces	7.242 (19.251)	-	-	-	0.0783 (0.016)	1.145 (3.190)	8.465
	CB-FAc	0.013 (0.007)	5.713 (5.135)	-	-	-	0.0156 (0.005)	2.082 (1.440)	7.824
G76	DB-FTi	-	18.027 (11.854)	-	-	-	0.233 (0.045)	2.728 (1.535)	20.988
	CB-FTi	Traces	-	-	-	-	0.0687 (0.009)	-	0.069
	CB-FAc	-	7.203 (6.800)	-	-	-	0.0186 (0.005)	2.055 (1.604)	9.277
G75A	DB-FTi	Traces	7.529 (17.676)	-	-	-	0.281 (0.086)	0.830 (1.662)	8.639
	CB-FTi	Traces	0.711 (1.401)	-	-	-	0.0774 (0.006)	-	0.789
	CB-FAc	Traces	1.447 (0.910)	-	-	-	0.0164 (0.006)	0.429 (0.444)	1.892
G75B	DB-FTi	Traces	6.785 (5.165)	-	-	-	0.268 (0.065)	1.107 (0.811)	8.160
	CB-FTi	Traces	3.457 (7.288)	-	-	-	0.0899 (0.016)	0.543 (1.213)	4.090
	CB-FAc	0.013 (0.006)	10.703 (9.489)	-	-	-	0.0135 (0.003)	1.888 (1.436)	12.617
G58	DB-FTi	0.061 (0.024)	14.986 (7.946)	-	-	-	0.218 (0.082)	0.849 (0.435)	16.113
	CB-FTi	0.040 (0.021)	13.923 (18.474)	-	-	-	0.0931 (0.008)	0.573 (0.734)	14.629
	CB-FAc	0.012 (0.014)	7.874 (3.318)	-	-	-	0.0163 (0.004)	0.389 (0.177)	8.291
GP	DB-FTi	0.024 (0.013)	0.525 (0.304)	Traces	0.381 (0.785)	-	0.270 (0.120)	0.121 (0.140)	1.320
	CB-FTi	0.482 (0.413)	10.634 (27.244)	0.039 (0.041)	0.447 (0.596)	-	0.0746 (0.020)	0.250 (0.734)	11.926
	CB-FAc	0.015 (0.008)	14.241 (9.381)	Traces	0.251 (0.329)	-	0.0161 (0.007)	0.542 (0.331)	15.065
SL	DB-FTi	55.571 (3.734)	-	-	34.557 (4.272)	3.246 (0.333)	0.201 (0.078)	14.129 (2.142)	107.704
	CB-FTi	14.014 (6.485)	-	-	18.871 (5.463)	1.310 (0.379)	0.1069 (0.140)	9.294 (3.180)	43.597
	CB-FAc	9.254 (0.796)	-	-	13.971 (1.746)	0.923 (0.087)	0.0145 (0.002)	6.494 (0.681)	30.658
SAc	CB-FTi	-	-	-	-	-	0.1500 (0.019)	-	0.150

Standard deviations in parentheses. - indicates below the detective limit.

Ga, In, and Sn, were released at high levels, and only Zn was released, other than Ti, from the combinations with C98. The released levels of these elements were higher in the combinations with titanium than with acryl. Regarding gold alloys, the levels of Cu and Zn release were high. The standard deviation of the Cu release level was the largest, showing a large variation. The number of elements released from GP was the largest. The combination showing the highest level of release was SL/DB-FTi. Ti release showed a specific tendency depending on the combined fixture and fixing method, as described below. Fig. 2 shows the levels of Ti release in the DB-FTi and CB-FTi groups. In the DB-FTi group, the Ti release level was lower in the combinations with dental alloys than with the same metal, STi. When these measured values were subjected to the Kruskal Wallis test, significant differences were noted ($p < 0.01$). In the cement-fixed CB-FTi group, no significant difference was noted in the Ti release level among the combinations. The level

of Ti release was markedly lower in the CB-FTi than in the DB-FTi group, and a markedly significant difference was detected on multiple comparison ($p < 0.001$).

Table 4 shows the levels of metal ions released from the combinations of the fixture FTi2 and superstructures (Ti2, C94, G76, GP, and SL) bound by direct fixation, DB-FTi2, and combinations of the fixture F64 and superstructures (S64, C94, G76, GP, SL, and STi) bound by direct fixation, DB-F64. Seven elements were released in the DB-FTi2 group and these were the same as those released from DB-FTi. V and a low level of Al were additionally detected in the DB-F64 group. The total level of metal release was markedly higher in the combinations with SL, as noted in the DB-FTi, CB-FTi, and CB-F64 groups. Fig. 3 shows a comparison of the Ti release level between the groups using FTi2 and FTi for fixtures bound by direct fixation, DB-FTi2 and DB-FTi, respectively. Contrary to the DB-FTi group described above, the level of Ti

Fig. 2 Levels of Ti release from DB-FTi and CB-FTi.

Fig. 3 Levels of Ti release from DB-FTi and DB-FTi2.

Table 4 Total levels of metal ion release from DB-FTi2 and DB-F64

Sample		Released ion concentratoion (ppm)									
Super-structure	Group	Ag	Al	Cu	Ga	In	Sn	Ti	V	Zn	Total
STi2	DB-FTi2	-	-	-	-	-	-	0.493(0.060)	-	-	0.493
C94		-	-	-	0.159(0.080)	2.451(0.574)	0.157(0.039)	0.604(0.150)	-	-	3.372
G76		-	-	3.447(7.602)	-	-	-	0.575(0.077)	-	0.729(1.409)	4.751
GP		0.019 (0.008)	-	1.148(1.761)	0.024(0.011)	0.064(0.079)	-	0.547(0.072)	-	0.166(0.204)	1.967
SL		20.299 (15.015)	-	-	-	13.253(9.993)	1.154(0.873)	0.489(0.113)	-	6.078(4.892)	41.273
S64	DB-F64	-	Traces	-	-	-	-	0.649(0.162)	0.031(0.007)	-	0.681
C94		-	Traces	-	0.325(0.250)	2.319(0.982)	0.117(0.052)	0.319(0.078)	0.016(0.004)	-	3.096
G76		-	Traces	0.885(0.860)	-	-	-	0.326(0.021)	0.017(0.001)	0.224(0.282)	1.451
GP		0.013 (0.012)	Traces	2.299(2.671)	Traces	0.041(0.080)	-	0.340(0.072)	0.017(0.003)	0.226(0.223)	2.937
SL		56.800 (12.309)	Traces	-	-	38.971(9.963)	3.141(0.725)	0.233(0.032)	0.009(0.002)	18.671(4.838)	117.827
STi		-	Traces	-	-	-	-	1.158(0.963)	0.037(0.031)	-	1.195

Standard deviations in parentheses. - indicates below the detective limit.

Fig. 4 Levels of Ti release from DB-F64.

Fig. 7 Discolorations of superstructures after immersion in DB-FTi, CB-FTi, and CB-FAc.

Fig. 5 Levels of V release from DB-F64.

Fig. 8 Discolorations of superstructures after immersion in DB-FTi2.

Fig. 6 Levels of Ti release from titanium materials and acrylic bound by cement fixation.
 CB-FAc (STi): Titanium superstructure (STi) and acrylic fixture (FAc) bound by cement fixation.
 CB-FTi (SAc): Acrylic superstructure (SAc) and titanium fixture (FTi) bound by cement fixation.
 CB-FTi2 (SAc): Acrylic superstructure (SAc) and titanium fixture (FTi2) bound by cement fixation.

Fig. 9 Discolorations of superstructures after immersion in DB-F64.

Fig. 10 Photographs of micro-structure of metals of titanium and titanium alloy used for experiments. a: STi, b: FTi, c: STi2, d: FTi2, e: S64, f: F64. Bar is 30 μ m.

release was higher in the combination with precious metals than in that with the same metal, STi, in the DB-FTi2 group. On comparison between the two titanium-titanium combinations, the Ti level was lower in DB-FTi2 than in DB-FTi, and the difference was highly significant on two-way layout ANOVA ($p < 0.001$).

Fig. 4 shows the level of Ti release from DB-F64 in which the fixture F64 was directly bound. No

significant difference was noted in the release level between the titanium alloy-titanium alloy and titanium alloy-dental alloy combinations. However, the level was higher in the combination with STi compared to those in the other conditions, and the difference was significant on the Kruskal Wallis test ($p < 0.01$). Fig. 5 shows the V release levels. The level of release showed a similar tendency to that of Ti: there were no marked

Fig. 11 Microscopic photographs of the occlusal surfaces of superstructures under all conditions using SL before and after immersion. a: SL (before immersion), b: DB-FTi, c: CB-FTi, d: CB-FAc, e: DB-FTi2, f: DB-F64. Bar is 30 μ m.

differences between the titanium alloy-titanium alloy and titanium alloy-dental alloy combinations, but the level was higher in the combination with STi compared to those in the other conditions, and the difference was significant on the Kruskal Wallis test ($p < 0.01$).

Fig. 6 shows the Ti levels released from the STi/ acrylic fixture (FAc) bound by cement fixation and acrylic superstructures (SAc)/ FTi and FTi2 bound by

cement fixation. On one-way layout ANOVA of these measured values, a markedly significant difference was noted between the titanium types ($p < 0.001$), and markedly significant differences were observed between FTi/STi and FTi2 on Tukey's multiple comparison ($p < 0.001$).

Measurement of discoloration after immersion

Fig. 7 shows the discoloration of superstructures in the DB-FTi, CB-FTi, and CB-FAc groups, Fig. 8 shows those in the DB-FTi2 group, and Fig. 9 shows those in DB-F64. The largest change was noted in the combination with SL regardless of the condition, and a markedly significant difference was detected on the Kruskal-Wallis test ($p < 0.001$). The discoloration of the F64/SL combination bound by direct fixation was the most marked (38.71 ± 2.73), followed by FTi/SL bound by direct fixation (30.20 ± 8.29). The color difference was 6 or less in all other samples not combined with SL.

Observation of micro-structure of metal

Fig. 10 shows photographs of micro-structure of metals of titanium and titanium alloy (STi: a, FTi: b, STi2: c, FTi2: d, S64: e, F64: f). The grain size varied among the titanium types used in this experiment, although all were JIS type 2, and the size decreased in the order of STi2, FTi2 > STi > FTi. The twin was observed in all the titanium samples. The grain size of titanium alloy was smaller than that of titanium.

Observation of surfaces

Fig. 11 shows microscopic photographs of the occlusal surfaces of SL superstructures which showed marked changes after immersion: a: before immersion, b, c, d, e, and f: DB-FTi, CB-FTi, CB-FAc, DB-FTi2, and DB-F64 after immersion, respectively. The photographs also show differences in the surface change after immersion depending on the combination. Discoloration occurred in all samples, and findings suggesting corrosion were observed. Marked corrosion was noted in DB-FTi, DB-FTi2, and DB-F64 prepared by direct fixation with titanium and titanium alloy.

DISCUSSION

The total release level was the highest in combination with SL regardless of the condition (Tables 3 and 4), which may have been due to the low corrosion resistance of SL because the maximum current density of SL is the highest due to its passivation¹⁹. The highest total release level was detected when SL alloy was bound with titanium and titanium alloy by direct fixation. As seen on the surface observation (Fig. 11), this release may have been due to galvanic corrosion. Dental alloys are considered to exhibit high-level corrosion resistance, but metal ion release *in vitro* and *in vivo* has been reported^{20–22}. It has also been reported that, in gold alloys with a basic composition of Au-Ag-Cu, corrosion occurred in Ag-rich regions followed by Cu-rich regions²³. Ag-rich and Cu-rich regions may be formed because Ag and Cu do not fuse, promoting local galvanic corrosion²⁴. Accordingly, the release level may have been higher in the combinations with all Cu-containing gold alloys and GP than in those with PFM alloys containing no Cu. From the PFM alloys, characteristic trace elements not contained in gold alloys, such as Ga, In, and Sn, were released. However,

the total release level was as low as that from titanium-titanium combinations under all conditions excluding the combination with Zn-containing C98, which may have been due to the fact that the corrosion potential (natural electrode potential) of PFM alloys is higher than those of other dental alloys¹⁹.

Differences in the Ti release level were small among the 3 types of titanium with different micro-structure of titanium bound with acryl by cement fixation, although the differences were significant (Fig. 6). Reportedly, because the corrosion of the metal often progresses from the crystal grain boundary²⁵, corrosion resistance is different from the actual experiment results although it is thought to be inferior because the percentage of the crystal grain boundary of the metal with small crystal grains is greater. On the other hand, the corrosion resistance of titanium is not altered through material histories, such as welding, processing, and heating²⁶, which is consistent with our findings. However, the level of Ti release was markedly influenced by the micro-structure of titanium in the metal/metal combinations (Fig. 3). The level of Ti released from FTi2 with a coarse-grained size was more than 2 times higher in the combinations with dental alloys than with acryl bound by cement fixation (Figs. 3 and 6). In contrast, Ti release from FTi with a fine-grained size increased in the combinations with dental alloys than with acryl bound by cement fixation, but to only about half of that released from FTi2 (Fig. 3). Furthermore, the highest Ti release level was detected in the combination of titanium with different micro-structure of titanium (Tables 3 and 4), suggesting that a condition similar to galvanic couple corrosion was formed between the same metal with different micro-structure of metal. Similarly, the release level from combinations with the titanium alloy fixture was significantly higher in the combination with titanium superstructure exhibiting electrochemical properties similar to those of titanium alloy¹⁹ than with titanium alloy cut out from the same wire and dental alloys (Fig. 4). These findings may also have been due to the difference in the micro-structure of metal between titanium alloy and titanium.

Kitamura *et al.*¹⁸ performed an electrochemical investigation, and observed that titanium/titanium and titanium alloy/titanium alloy combinations were superior to titanium/titanium alloy and titanium/Au-Ag-Pd alloy combinations in terms of corrosion resistance. In addition, Foti *et al.*²⁷ reported that Ti was detected in bone around titanium fixtures when dental alloys were used for superstructures, while no Ti was detected when titanium was used for superstructures in an animal experiment. On the other hand, Reclaru *et al.*¹³ and Grosogeat *et al.*²⁸ investigated galvanic corrosion, and found that the galvanic current was very low in titanium/precious metal combinations, having only a small influence on corrosion. The previously reported inconsistent data on Ti release described above^{13,18,27,28} may have been associated with the micro-structure of titanium, and

consideration of the micro-structure of metal of titanium and titanium alloys may be necessary to consider combinations of titanium and titanium alloys with other metals. This may also similarly apply to V release, which showed a similar tendency to that of the Ti release level (Fig. 5). Ti release at 0.0135–0.0216 ppm was detected in the combinations with CB-FAc containing no Ti (Table 3). To confirm this finding, the cement alone was immersed in 1% lactic acid and subjected to ICP analysis. Al, B, Ga, Fe, Mg, Si, Ti, and Zn were detected, suggesting that the Ti released was derived from the cement. Similarly, about 0.01–0.02 ppm of Ti released from cement-fixed CB-FTi may have been derived from cement.

Regarding the biological influences of released metals, the maximum Ag release during the 3-month immersion was 56.8 ppm from DB-F64 (SL) (Table 4). Inoue *et al.*²⁹⁾ reported that Ag is unlikely to cause allergy because the positive Ag sensitization rate is the lowest, being only 0.1%, in both healthy and morbid persons, suggesting that the risk is low. Cu is an essential trace element in humans, and the estimated average requirements are 0.6–0.7 and 0.5–0.7 mg/day for adult men and women, respectively, and the tolerable upper intake level is 10 mg/day³⁰⁾. The maximum Cu release during 3-month immersion was 21.2 ppm from DB-F64 (G77), suggesting that the risk is low. Regarding Ga, the 50% lethal dose (LD50) of cytotoxicity for mouse fibroblasts has been reported to be 0.251 ppm of Ga³⁺ (GaCl₃; 3.60×10^{-5} mol L⁻¹)³¹⁾. The maximum Ga release during the 3-month immersion was 0.295 ppm from DB-F64 (C94), suggesting that the risk is also low. Regarding In, Inoue *et al.*²⁹⁾ reported positive sensitization rates of 0.4 and 2.8% in healthy and morbid persons, respectively. The maximum In release during the 3-month immersion was 39.0 ppm from DB-F64 (SL), for which the risk of allergy cannot be neglected, although the positive sensitization rate in morbid persons is lower than those for other metals. Materials containing no In should be selected for persons with In allergy. Inoue *et al.*²⁹⁾ also reported that the positive Sn sensitization rates were 6.3 and 13.3% in healthy and morbid persons, respectively. The maximum Sn release during 3-month immersion was 56.8 ppm from DB-FTi (SL), which may involve a risk of allergy even in healthy persons. Zn is also an essential trace element for humans, and gustatory disorder is reportedly a typical symptom of Zn deficiency. The estimated average requirement and tolerable upper intake level are 9–10 and 40–45 mg/day for adult men and 7–8 and 30–35 mg/day for adult women, respectively³⁰⁾. The positive Zn sensitization rates were reported to be 1.7 and 7.3% in healthy and morbid persons, respectively²⁹⁾. The maximum Zn release during the 3-month immersion was 21.2 ppm from DB-F64 (SL), suggesting that the risk may be low for healthy but high for morbid persons. Materials containing no Zn should be selected for those with Zn allergy. Titanium shows high-level corrosion resistance due to the passive surface capsule and biocompatibility,

but Fujimoto³²⁾ reported that the mouse fibroblast colony formation rate was 0% in the presence of 20 ppm of Ti⁴⁺ on a cytotoxicity test, showing its toxicity. The maximum Ti release during the 3-month immersion was 1.4 ppm from DB-FTi (STi), and the colony formation rate may be 99–84% based on the findings reported by Fujimoto, suggesting that the toxicity is low. Roppongi *et al.*³³⁾ implanted a test fragment consisting of pure titanium contacted with Co-Cr alloy in rabbits and found that Co release was promoted, suggesting that the combination of titanium with Co-Cr alloy is inappropriate because the positive Co sensitization rate is high. Meachim *et al.*³⁴⁾ reported that Ti and Fe, which are unstable as ions, are likely to accumulate in the surrounding tissue. Moreover, titanium and titanium alloy allergies have recently been reported^{35,36)}. On the other hand, the negativity of patients with titanium hypersensitivity on patch and intracutaneous tests with titanium salts has been reported, and the usefulness of *in vitro* lymphocyte stimulation and transformation tests have been investigated^{37,38)}. Okazaki *et al.*³⁹⁾ reported that the stress region of titanium and its surroundings serve as an anode and cathode under frictional conditions, respectively, and promote corrosion compared to that under static conditions. Moreover, Kumazawa *et al.*⁴⁰⁾ reported that titanium particles induced cytotoxicity. These reports suggest that the investigation of titanium particles which may be produced by friction is necessary in order to use titanium as a material for the occlusal surface. Although titanium and titanium alloys are considered to be superior in terms of corrosion resistance and biocompatibility, and criteria for use in implants have been established, corrosion and influences on biological tissue have been reported, as described above.

It was suggested that cement fixation is appropriate for the binding of superstructures with fixtures because it was less influential on the binding of different alloys and titanium materials with different micro-structure of metal with regard to the level of Ti release. Concerning metals for superstructures, PFM alloys may be suitable because the total metal ion release level was low. In titanium and titanium alloy implant systems composed of two or more pieces, it was thought that it was preferable that those micro-structures of metal were similar. Furthermore, a small grain size of Ti may be favorable for fixtures because the levels of Ti released from combinations with dental alloys were low.

CONCLUSION

Superstructures prepared with titanium, titanium alloy, dental alloys, and acryl were bound with titanium or titanium alloy fixtures by cement or direct fixation, and these test samples were immersed in 1% lactic acid solution for 3 months. The released metal ion levels and discoloration of superstructures after immersion were measured, and titanium and titanium

alloy tissues and the surfaces of the test samples before and after immersion were observed. The following conclusions were made:

1. The Ti release level was significantly lower in cement-fixed titanium/titanium and titanium/dental alloy combinations than in those directly fixed.
2. The Ti release level from titanium/dental alloy combinations was influenced by the micro-structure of titanium, and the level of release decreased when the titanium grain size was small.
3. In titanium/titanium combination, differences in the micro-structure of metal markedly influenced the release, and the level of release increased when the micro-structure of titanium was different.
4. Regarding the combination with titanium alloy, the Ti and V release levels were higher when titanium alloy was used in combination with titanium than with titanium alloy and dental alloy.

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