Basic 13
Micro-Nano Surface Technology
– Bioactive Coating and Its Osteoconductivity –

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Contents

- Background & objective
- Hydro-coating process, (hydroxyapatite and titania)
  Pyro-process (titania)
    - Thermal substrate method
    - Anodizing
    - Immersion in oxidizing acid solution
    - High temperature oxidation
- Osteoconductivity evaluation by in vitro and in vivo testing.
- Conclusions

Background & Objective

Bio-*inert* Compound : Ti Alloy, Stainless Steel, ...

Combination

Bio-*active* Compound : **Hydroxyapatite, TiO_2, $CaTiO_3$,...**

High / Slow ← **strength / healing rate** → **Low (Brittle) / Rapid**

Bio-active Compound Coating on Metallic Implants

Coating Process

Many methods were proposed
to form the Bio-active Compound Coating

HAp	**Thermal Substrate Method**	**Plasma Spray**, Sol-Gel, ...
TiO_2	**Anodizing in Aqueous Solution** **Immersion in Oxidizing Solution**	**High Temperature Oxidation (Sinter)**
	Hydro-process	**(including) Pyro-process**

? Influence on the osteoconductivity of Coating Process

Coating

? Influence on the osteoconductivity of Coating Process

	Hydro-process	(including) Pyro-process
HAp	Thermal Substrate Method	**Plasma Spray**, Sol-Gel, ...

→ **HAp, CO_3Ap, CO_3Ap/$CaCO_3$, HAp/collagen, HAp/gelatin**

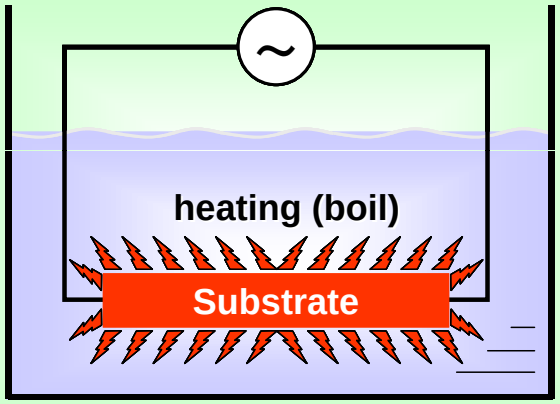
- **surface morphology**
- **coexistence of $CaCO_3$, collagen or gelatin**
- **CO_3 content collagen content gelatin content**

TiO_2	**Anodizing in Aqueous Solution** **Immersion in Oxidizing Solution**	**High Temperature Oxidation (Sinter)**

HAp Coating Theory

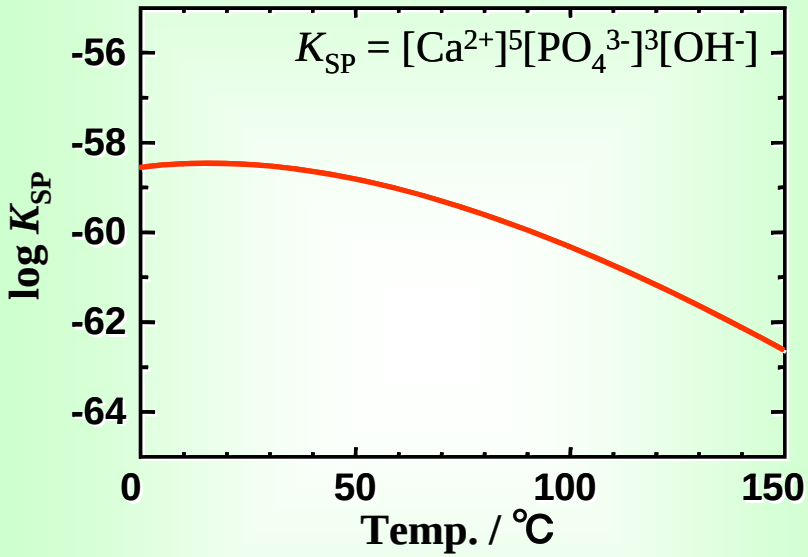
【 Thermal Substrate Method 】

An alternating current passes through a metallic sample immersed in an aqueous solution.



A Metallic sample in the aqueous solution heats up by Joule heating.

【 HAp precipitation】



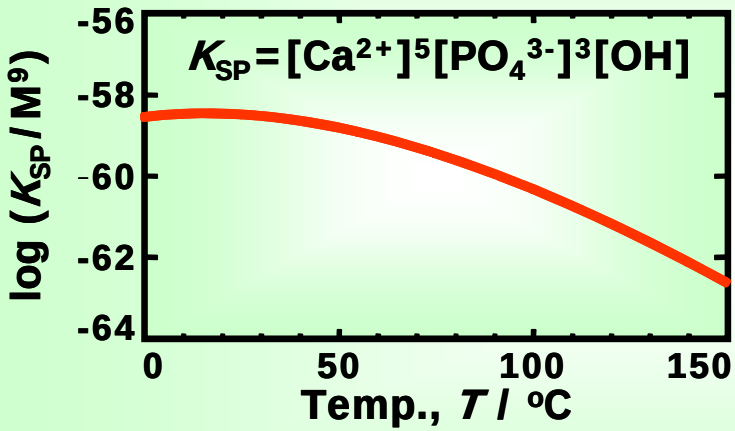
The solubility of HAp decreases with increasing temperature.

Combining “thermal substrate method” with HAp property can provide with the HAp coating on the heated substrate.

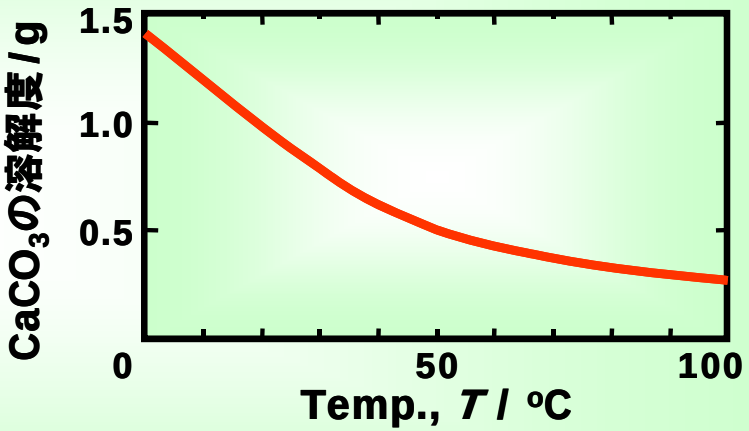
K. Kuroda, R. Ichino, M. Okido, O. Takai: J. Biomed. Mater. Res., Vol. 59, No. 2, p. 390-397, (2002)

CaCO3/CO3Ap Coating Theory

【 HAp precipitation】

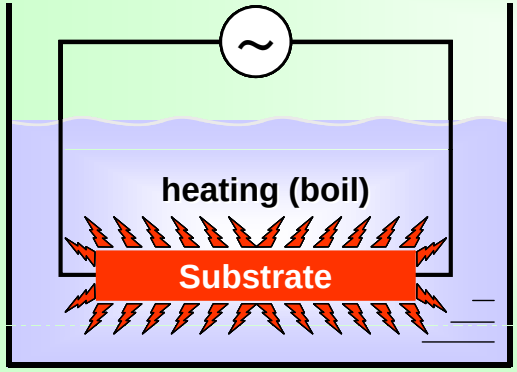


【 $CaCO_3$ precipitation】



The solubility of HAp & $CaCO_3$ decreases with increasing temperature.

【 Thermal Substrate Method 】



- A Metallic sample in the aqueous solution heats up by Joule heating.

HAp (CO_3Ap) and/or $CaCO_3$ precipitation on heated substrates by thermal substrate method.

Experimental

Condition

【Substrate】 **Ti plate** (0.3 ^{t} mm) for *in vitro*
Ti rod (ϕ2x5mm) for *in vivo*
(polished in SiC (#400))

【Solution】

0.7 mM $CaCl_2$
0.3 mM $Ca(H_2PO_4)_2$
pH 6~9 (adjusted by NaOH)
0.5~20 mM $NaHCO_3$ for CO_3Ap, $CaCO_3$/CO_3Ap
30~432 mg L^{-1} Collagen (Type I) for HAp/Col.

【Temp. (Substrate)】 **40~150 ºC**

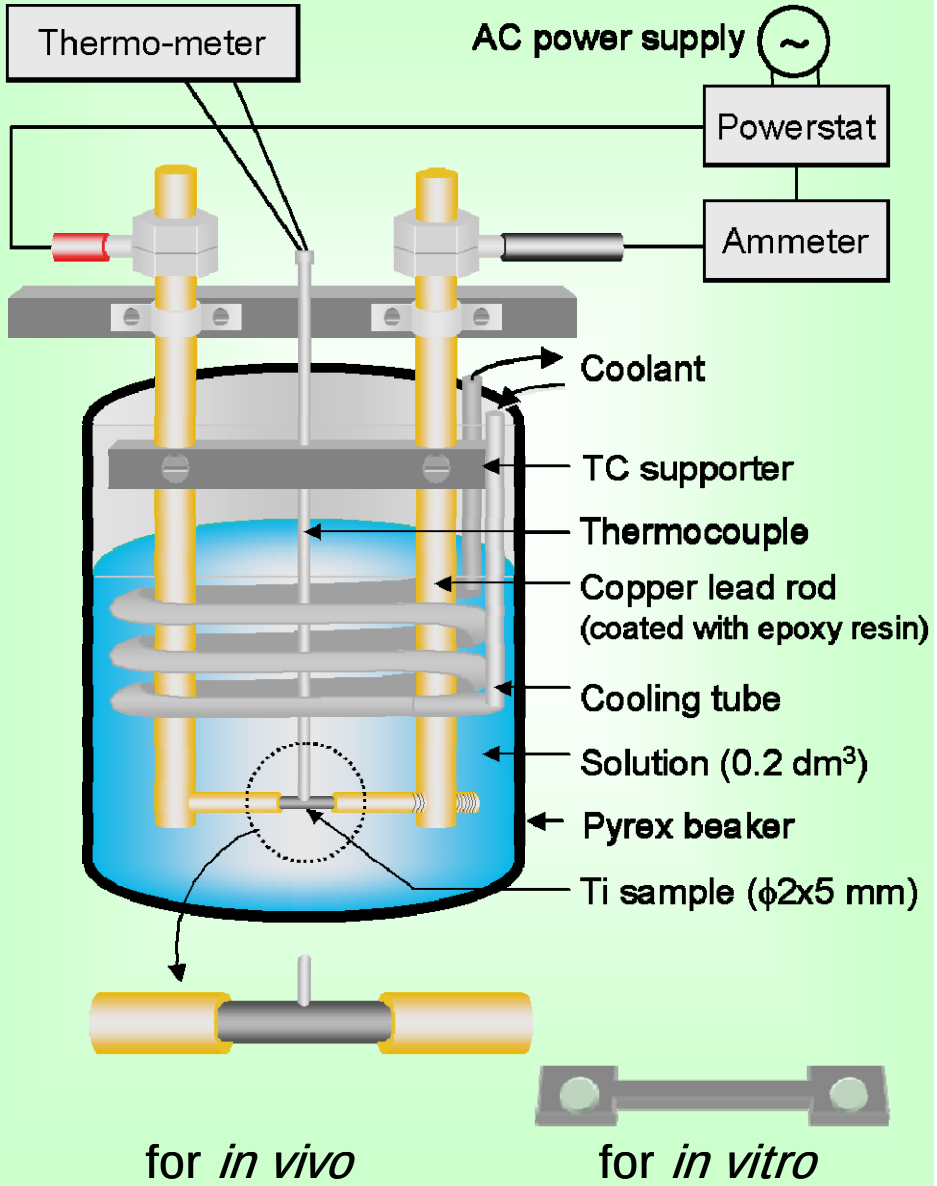
【Time】 **15~30 min.**

1 M **ethanol** addition & **ultrasonic wave** (at 40, 60 ºC)

Evaluation

SEM-EDX, XRD, FT-IR

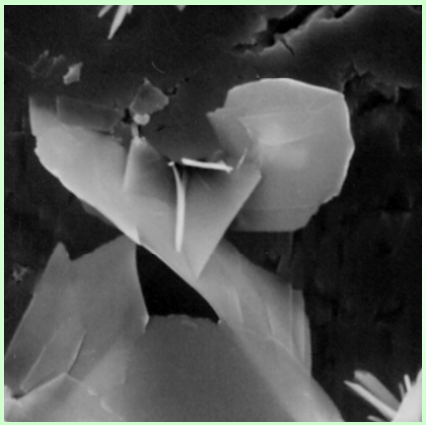
Apparatus



Effect of pH (for HAp Coating)

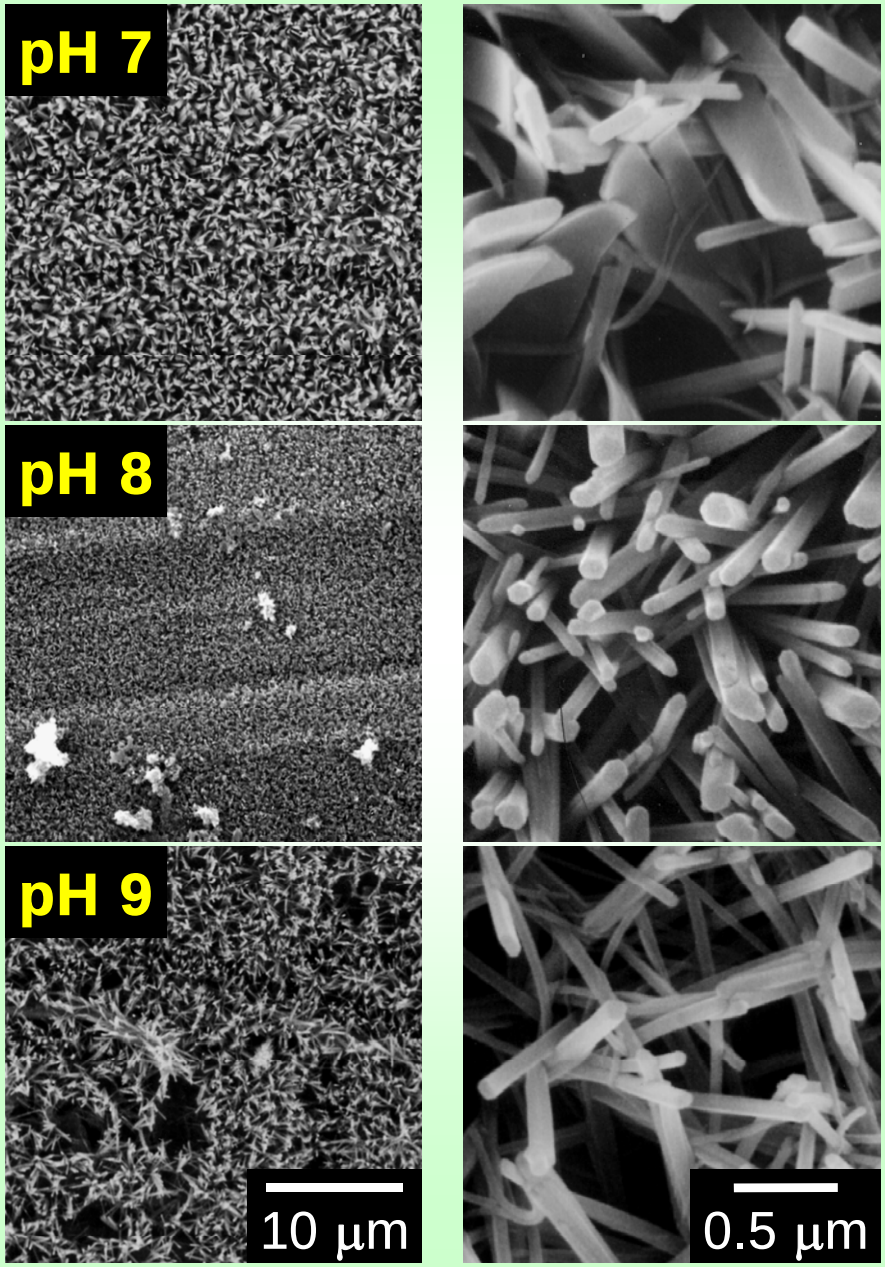
0.7mM $CaCl_2$, 0.3mM $Ca(H_2PO_4)_2$
heating time 20 min.
Temp. 150 °C
pH: adjusted by NaOHaq

- **Needle-like HAp was formed in the high pH range.**



pH 6

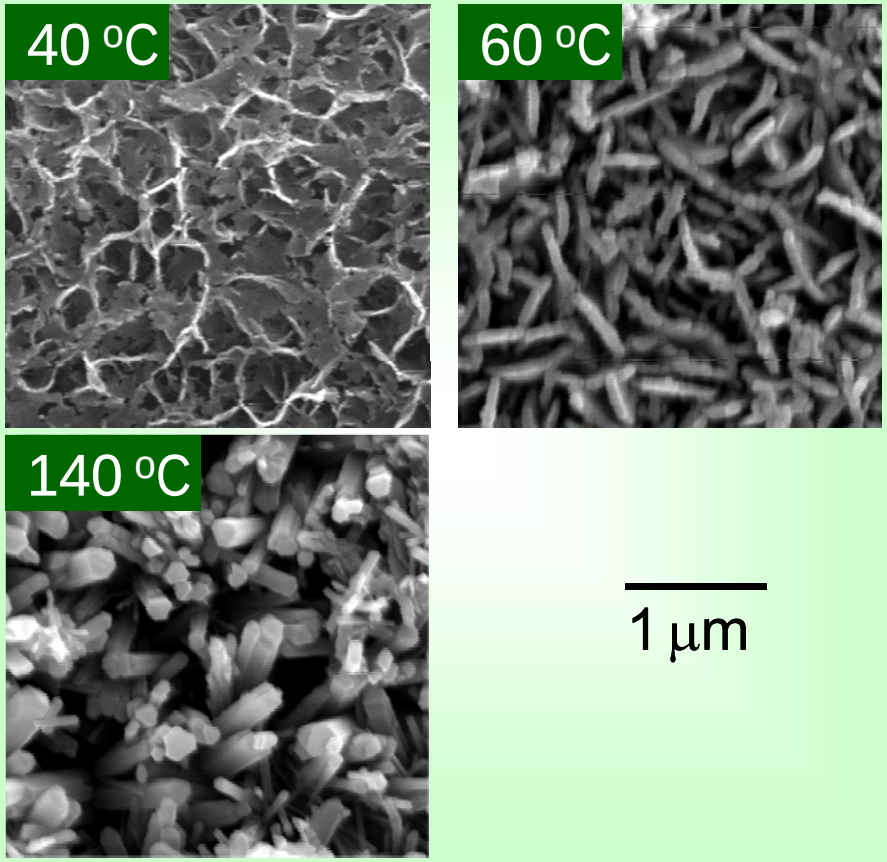
10 μm

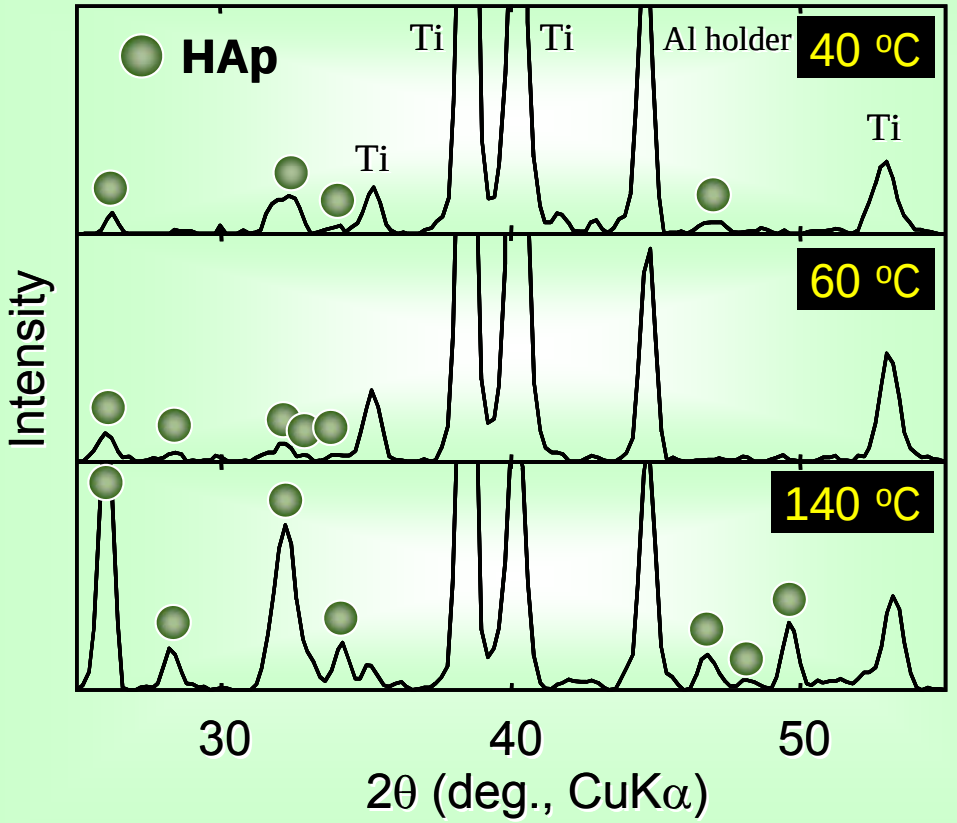


K. Kuroda, R. Ichino, M. Okido, O. Takai: J. Biomed. Mater. Res., Vol. 61, No. 3, p. 354-359, (2002)

Effect of Temp. (for HAp Coating)

0.7mM $CaCl_2$, 0.3mM $Ca(H_2PO_4)_2$, pH 8, 15 min.
(40 ºC & 60 ºC: 1M EtOH, ultrasonic wave(100kHz), 30 min.)

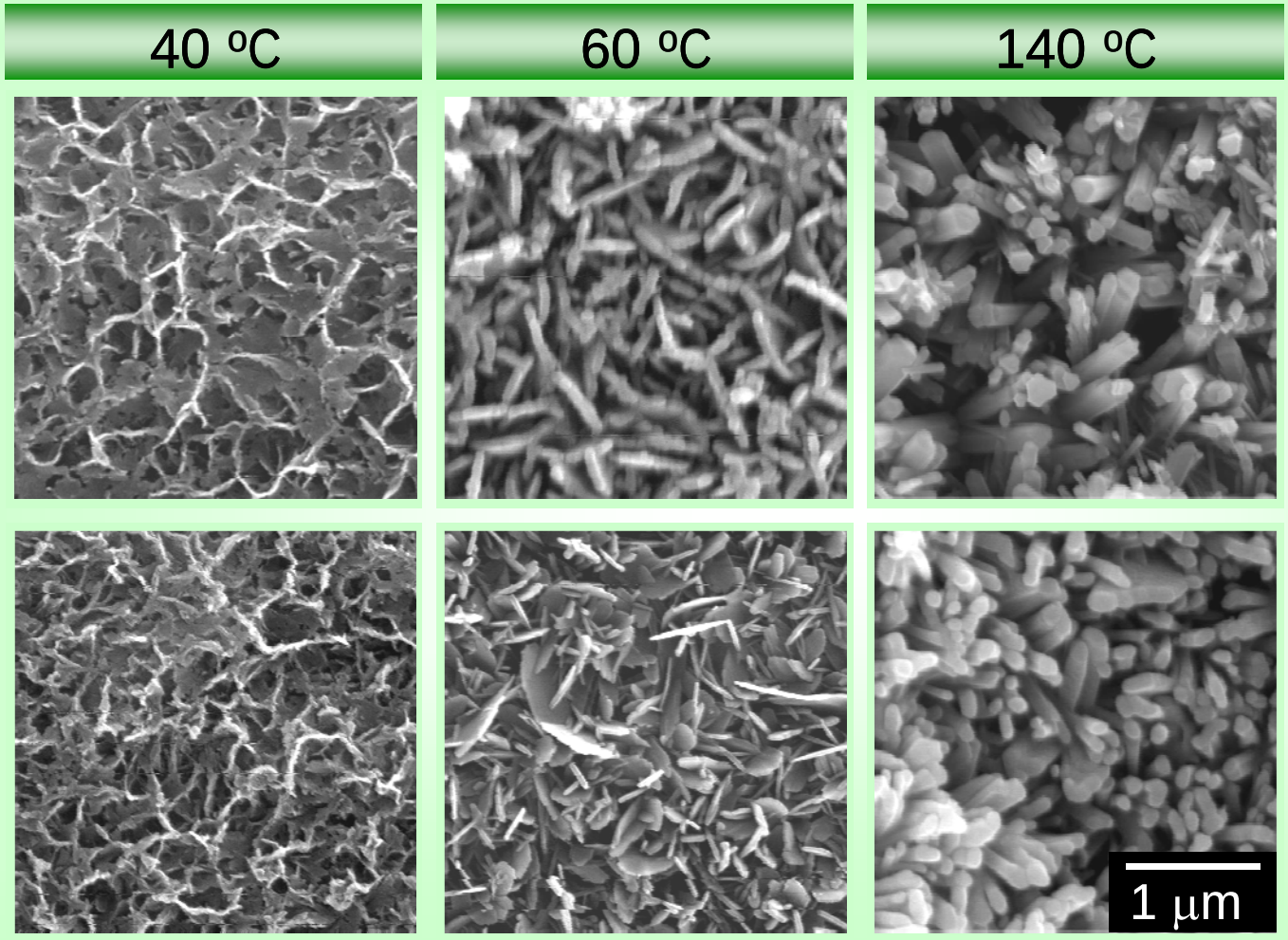




- **The surface morphology of HAp strongly depended on coating temperature; low temp. coating (40 ºC) net-like HAp, high temp. (140 ºC) needle-like, and mid temp. (60 ºC) plate-like .**

K. Kuroda, S. Nakamoto, Y. Miyashita, R. Ichino, M. Okido: Mater. Trans., Vol. 47, No. 5, p. 1931-1934,(2006)

Before in vitro & in vivo Evaluation



0.7mM $CaCl_2$
0.3mM $Ca(H_2PO_4)_2$
pH 8
15 min. (140 ºC)
30 min.
(40 & 60 ºC)

at 40 ºC & 60 ºC:

1M EtOH
ultrasonic wave
(100 kHz)

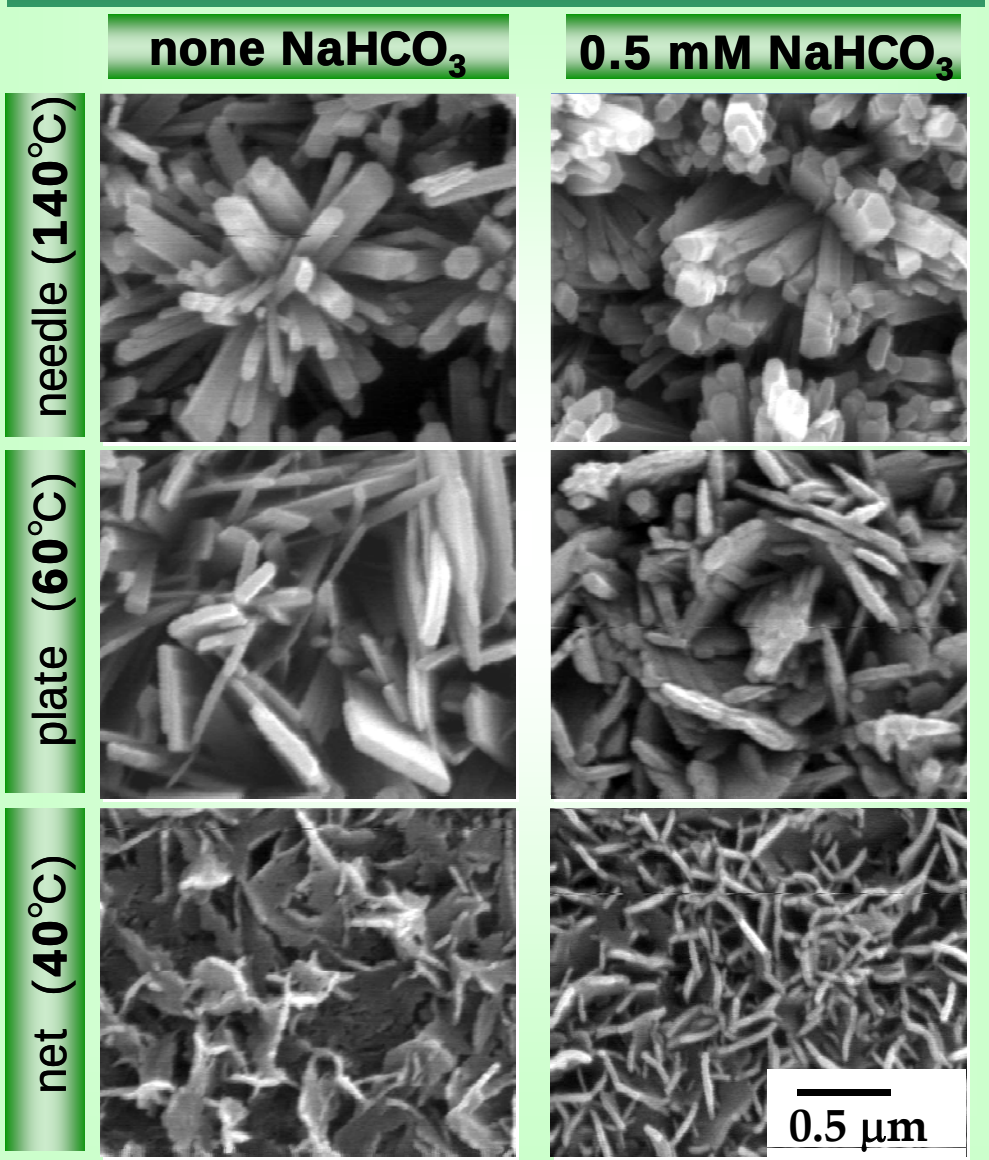
- **Direct exposure to the high temperature steam in the sterilizer caused *NO* changes in the surface morphologies.**
- **Therefore, after the sterilization, all the coated specimens were subjected to *in vitro* & *in vivo* testing.**

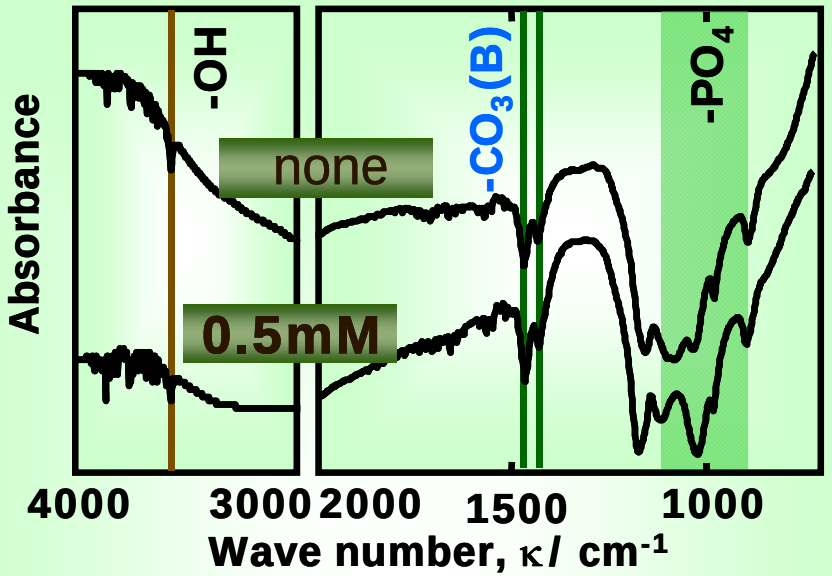
K. Kuroda, S. Nakamoto, Y. Miyashita, R. Ichino, M. Okido: Mater. Trans., Vol. 47, No. 5, p. 1931-1934,(2006)

CO3Ap/CaCO3 Coating

0.3 mM Ca(H2PO4), 0.7 mM CaCl2,,pH 8
(40 oC, 60 oC: 1 M ethanol addition, UW(100 kHz))

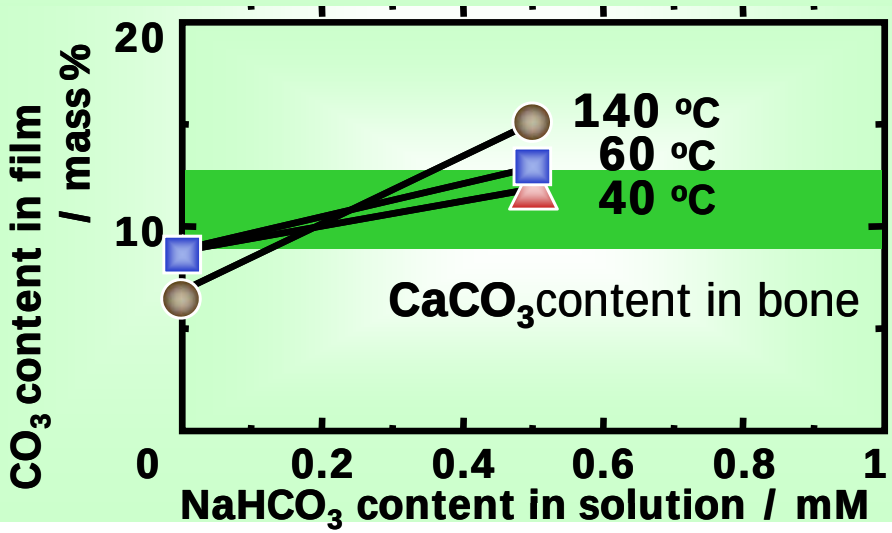
$NaHCO_3$ addition





Type B CO_3Ap CO_3^{2-} was substituted for PO_4^{3-}

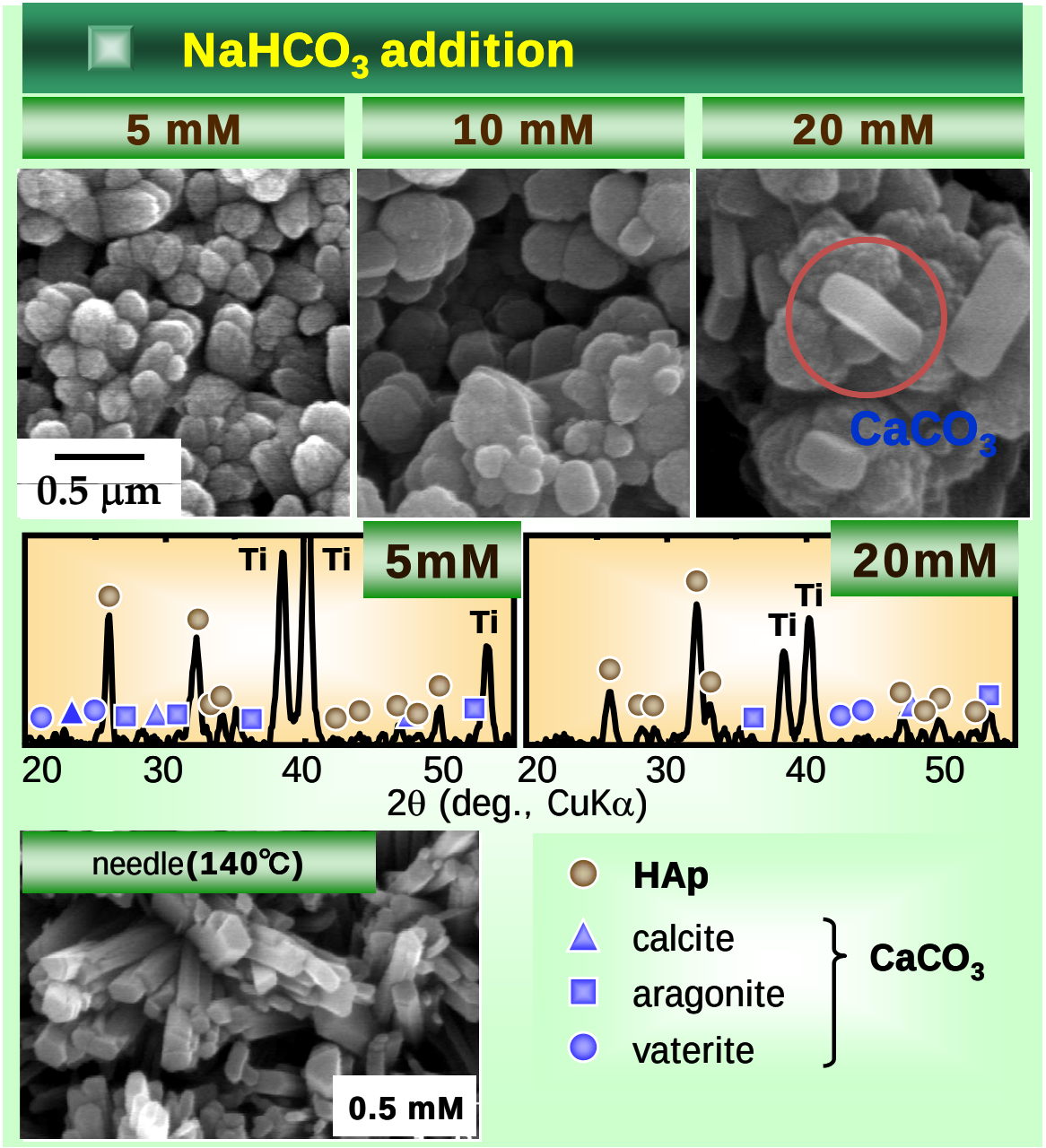
$$Ca_{10-y/2}[(PO_4)_{6-y}(CO_3)_y](OH)_2$$

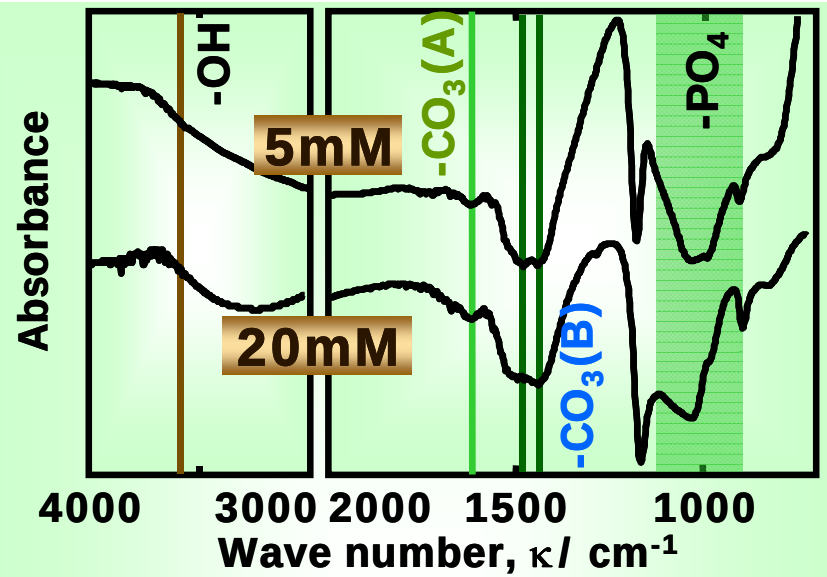


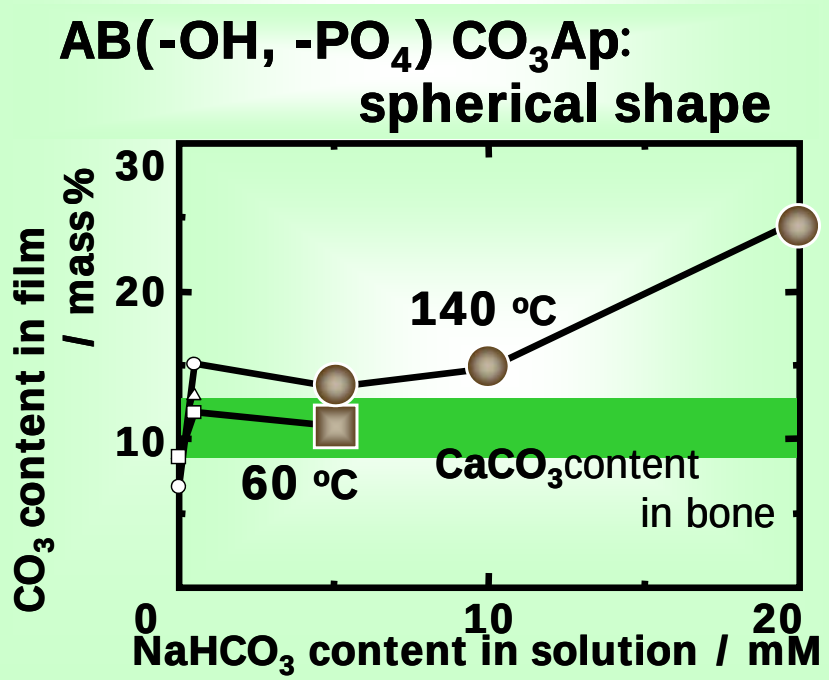
K. Kuroda, M. Moriyama, R. Ichino, M. Okido, A. Seki: Mater. Trans., Vol. 49, No. 6, p. 1434-1440, (2008)

CO3Ap/CaCO3 Coating

0.3 mM Ca(H2PO4), 0.7 mM CaCl2, pH 8
140 oC, 15 min.



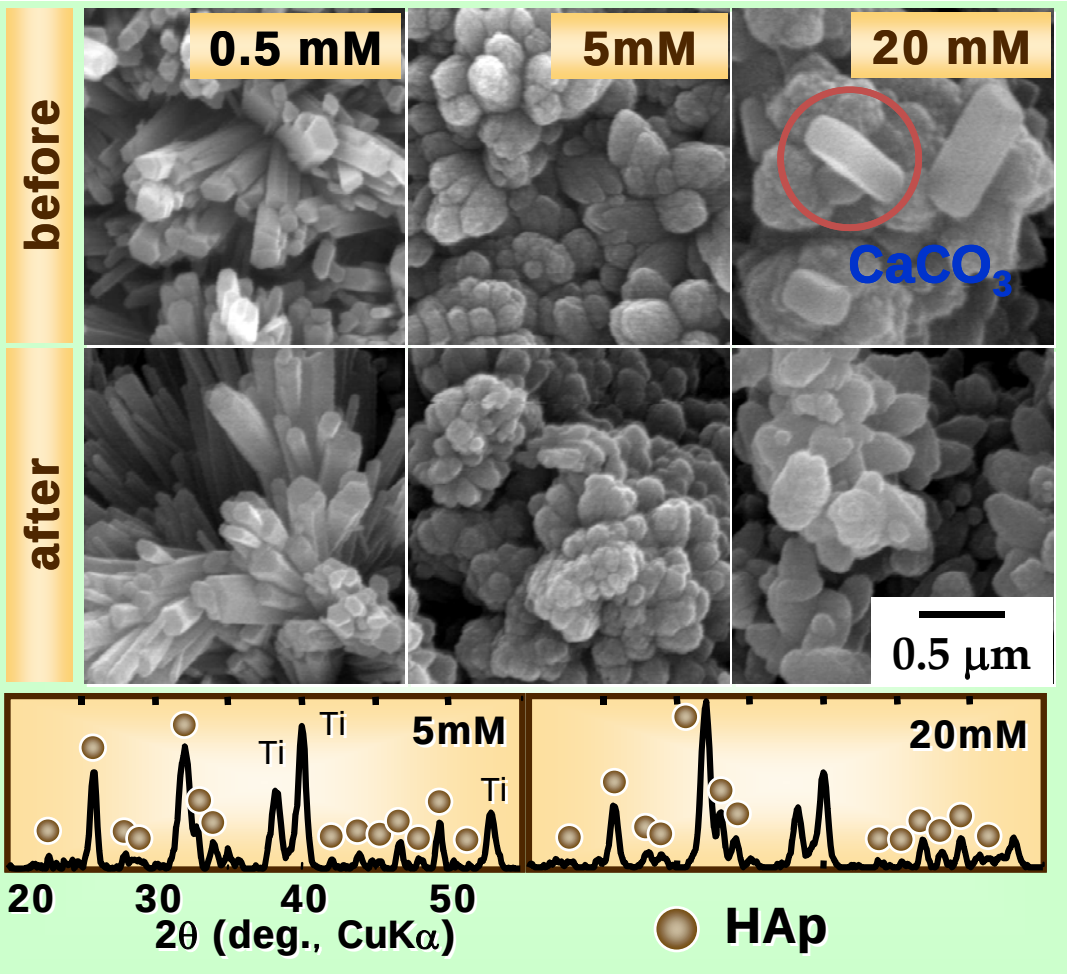


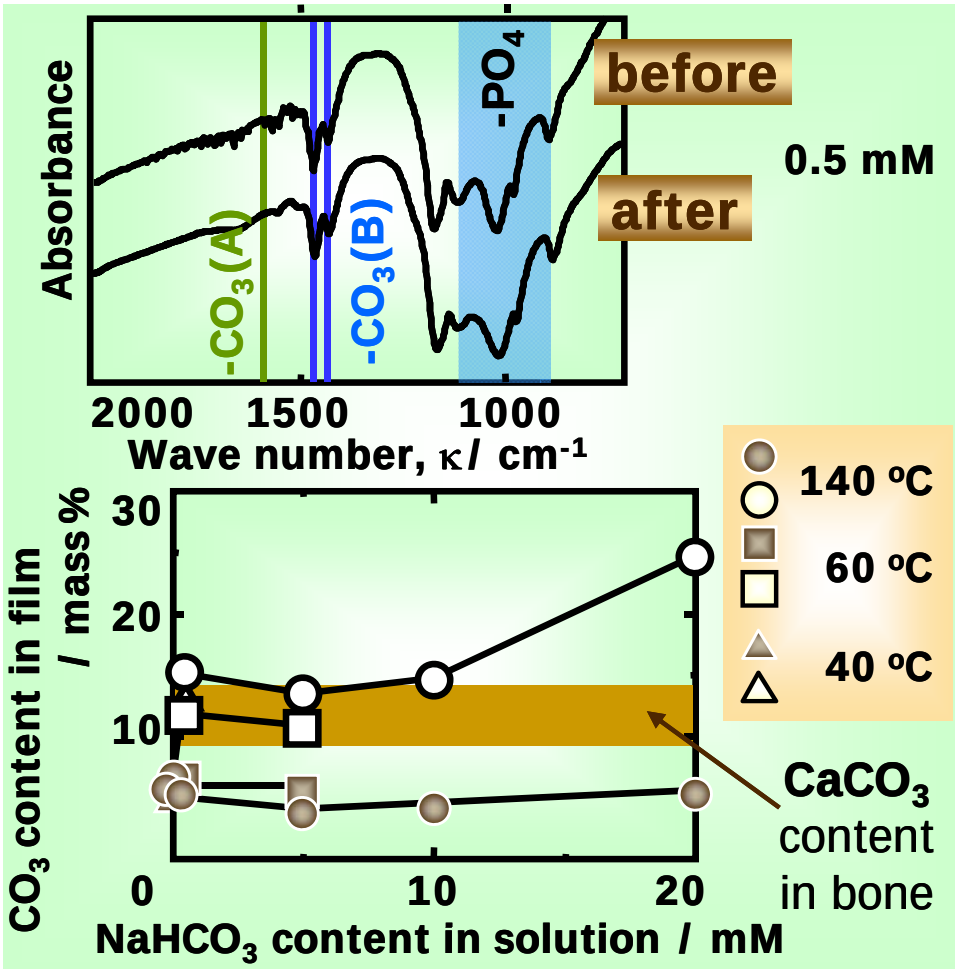


K. Kuroda, M. Moriyama, R. Ichino, M. Okido, A. Seki: Mater. Trans., Vol. 49, No. 6, p. 1434-1440, (2008)

After Autoclaving (CaCO3/CO3Ap Coating)

CO_3Ap coating: 0.3 mM $Ca(H_2PO_4)$, 0.7 mM $CaCl_2$, pH 8, 140 ºC, 15 min.
autoclaving: 121 ºC, 15 min.





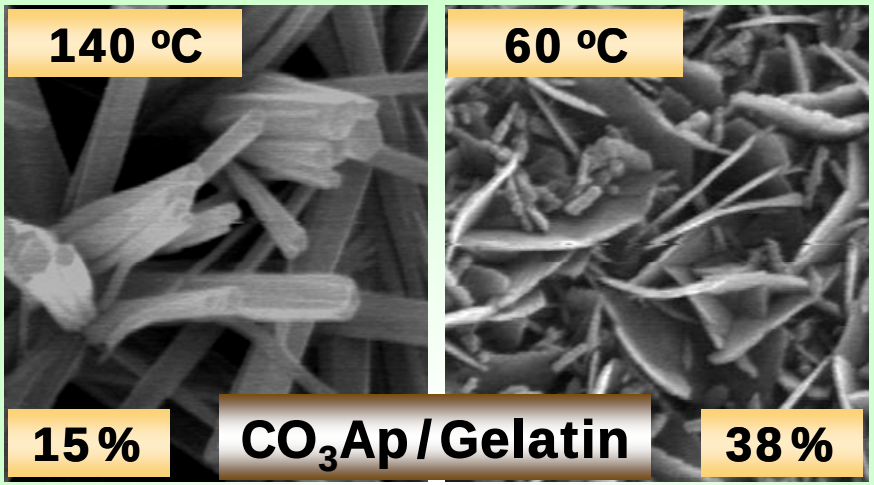
After autoclaving,
- **$CaCO_3$ disappeared, CO_3Ap (type B) remained.**
- **CO_3 content in coating was less than 5 mass%**

K. Kuroda, M. Moriyama, R. Ichino, M. Okido, A. Seki: Mater. Trans., Vol. 49, No. 6, p. 1434-1440, (2008)

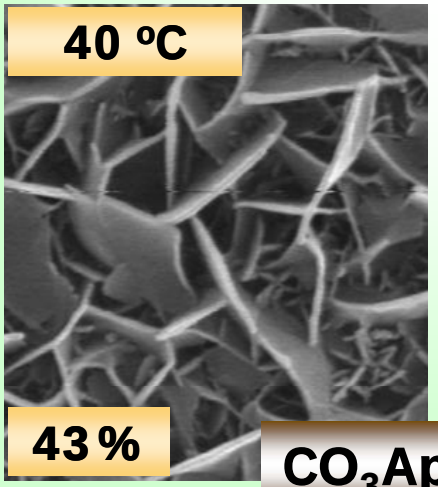
HAp/col. Coating

0.3 mM Ca(H2PO4), 0.7 mM CaCl2, pH 8
40~140 oC, collagen 30~432 mg L-1

Collagen addition



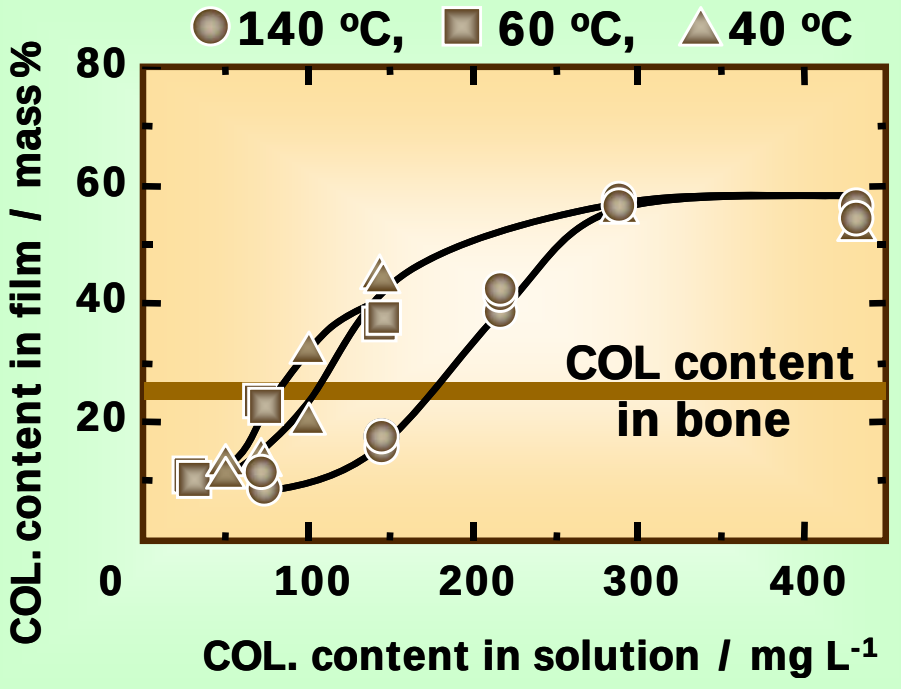
denaturalization of COL

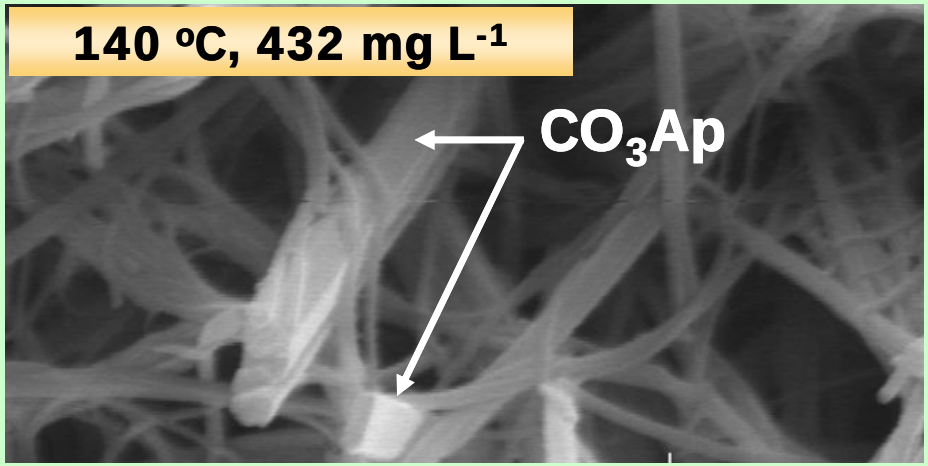


0.5 μm

COL content:
144 mg L-1

not denaturalization of COL

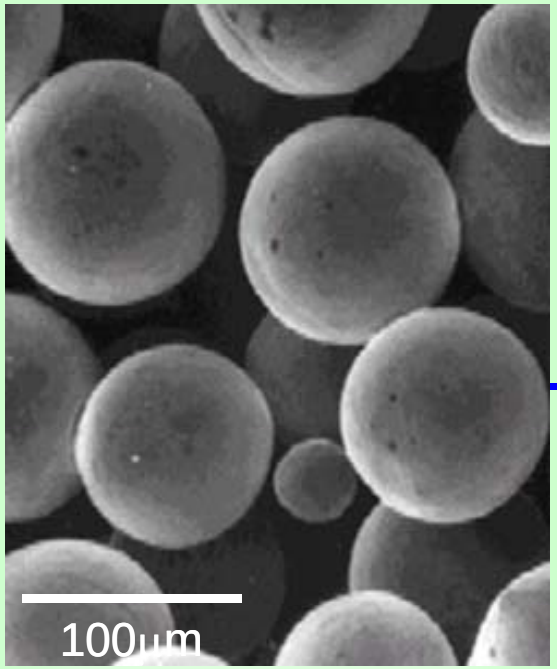




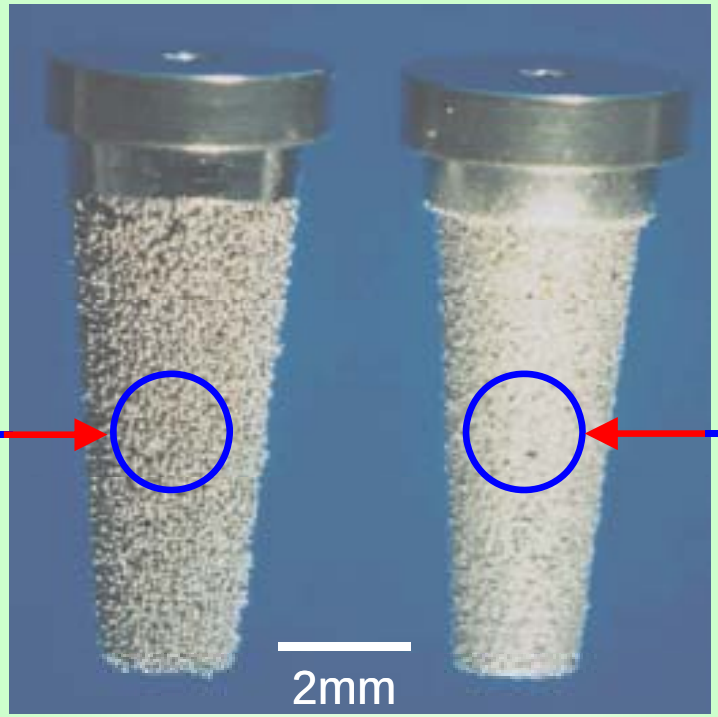
K. Kuroda, M. Moriyama, R. Ichino, M. Okido, A. Seki: Mater. Trans., Vol. 50, No. 5, p. 1190-1195, (2009)

Porous Surface

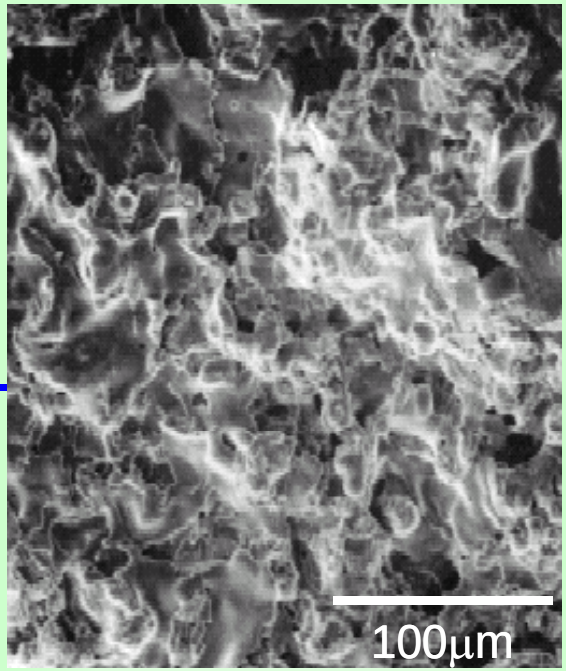
The objective of using beads sintered substrate is to fix implanted biomaterials on bone by inducing natural bone.



3D porous surface
(Ti-6Al-4V)



Tooth Root (Ti-6Al-4V)
Prof. R. M. Pilliar (Univ. Toronto, CA)

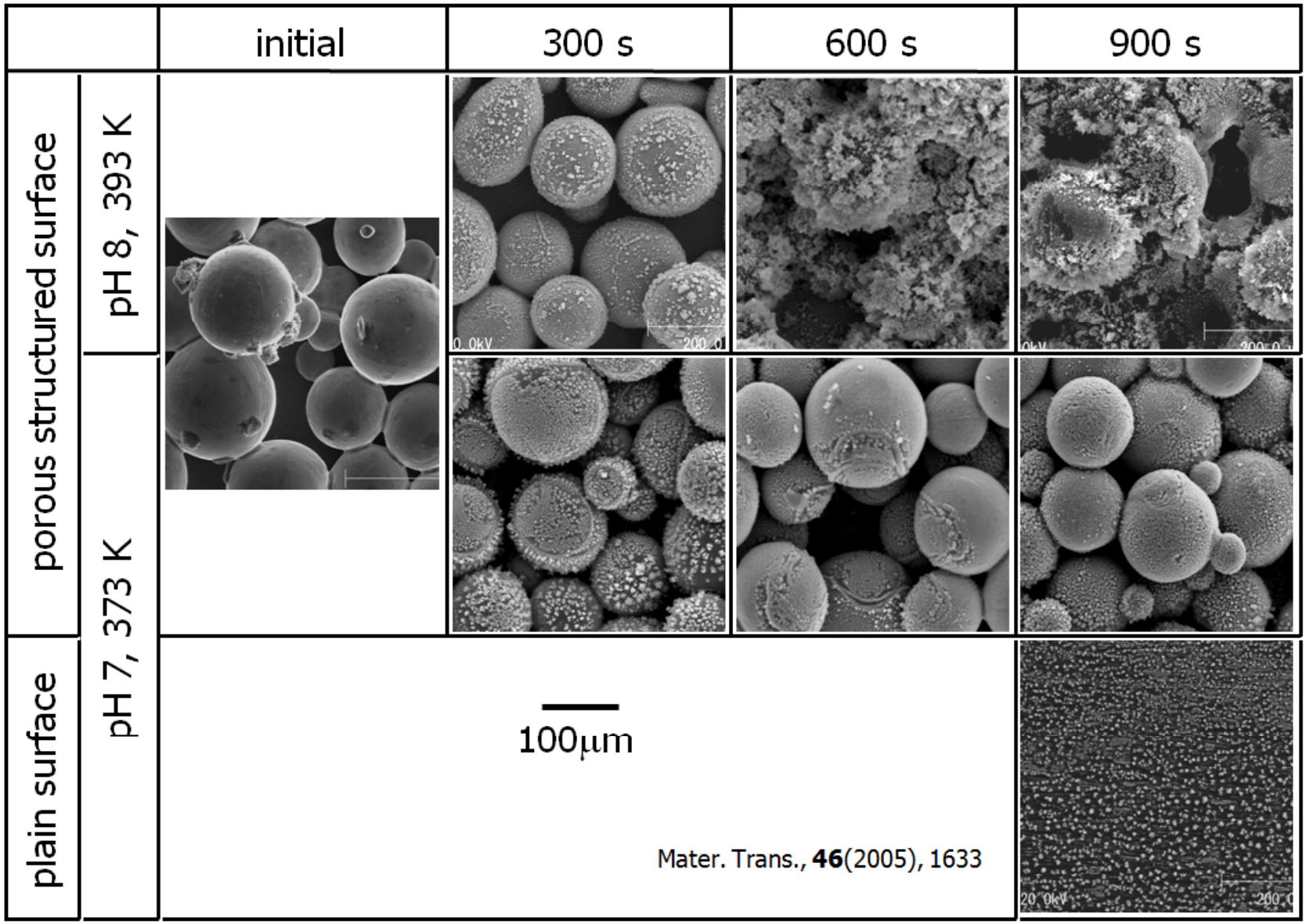


Plasma spray coating
on 3D porous surface

Plasma spray method can not maintain porous surface.

Can I coat HAp on Beads sintered Substrate?

【Thermal substrate method】 0.3 mM $Ca(H_2PO_4)_2$, 0.7 mM $CaCl_2$



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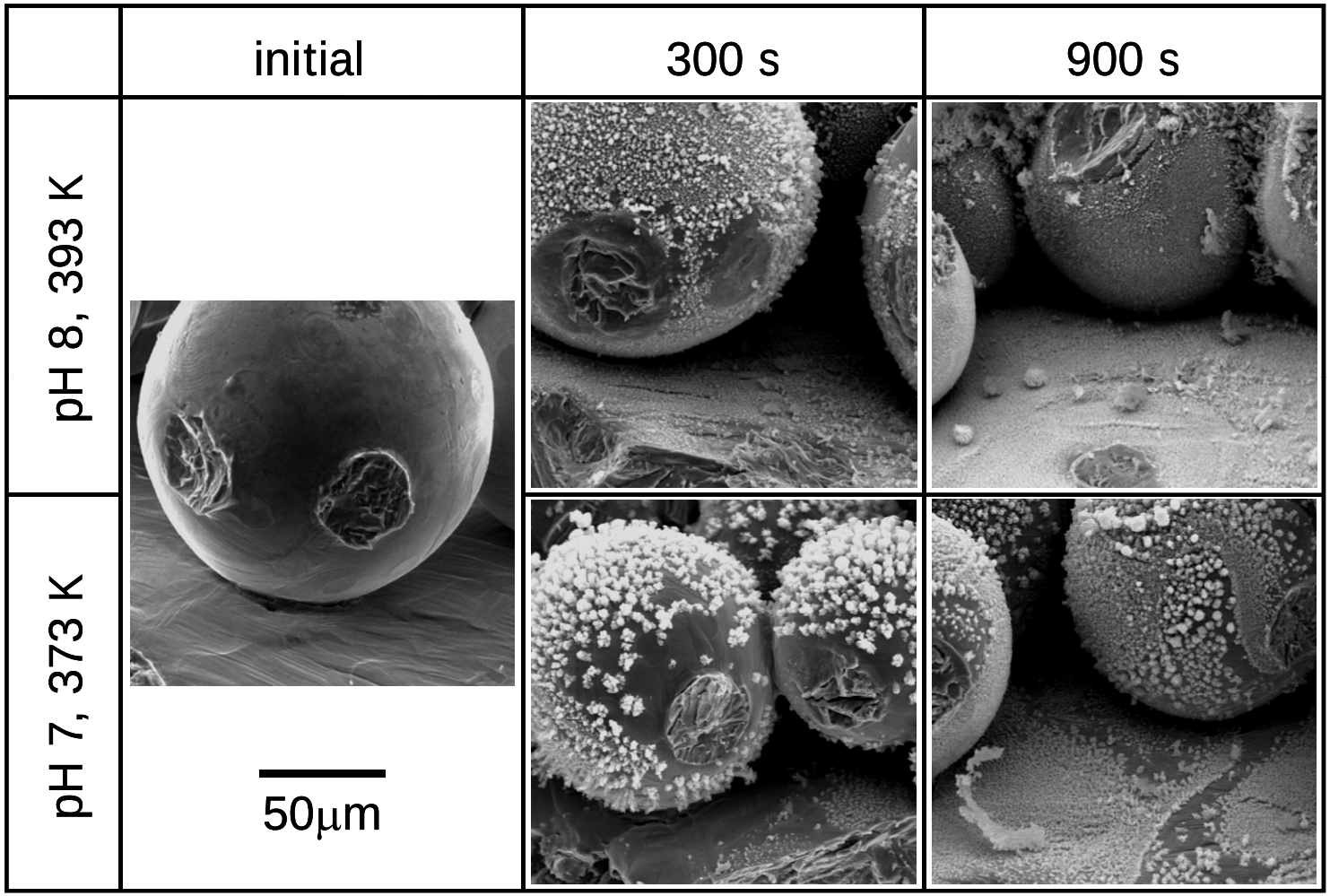


HAp precipitated also on the bottom of beads and the substrate.

Coating

? Influence on the osteoconductivity of Coating Process

	Hydro-process	(including) Pyro-process
HAp	Thermal Substrate Method	**Plasma Spray**, Sol-Gel, ...
TiO_2	Anodizing in Aqueous Solution Immersion in Oxidizing Solution	High Temperature Oxidation (Sinter)

→ anatase, rutile, (TiO)

- surface morphology (surface roughness)
- existence of anion and/or cation in coating
- crystallinity

Titania coating process

- Anodizing in Aqueous Solution
- Immersion in Oxidizing Solution
- High Temperature Oxidation

Anodizing in Aqueous Solution

Anodic electrolysis of Ti in aqueous solution

overall reaction: $Ti + 2H_2O \rightarrow TiO_2 + 4H^+ + 4e^-$

less than sparking E: **anatase,** more than: **anatase+rutile**

High Temperature Oxidation

Heating of Ti in gas phase

$Ti + O_2 \rightarrow TiO_2$ $(Ti + 1/2O_2 \rightarrow TiO)$

anatase→rutile :915 ºC
brockite→rutile :650 ºC

low temperature heating (400 ºC): **rutile** (Anatase is not obtained.)

Immersion in Oxidizing Solution

Immersion of Ti in the oxidizing acid solution containing H_2O_2 (more than 60 ºC).

low crystallinity **anatase** (hydrous gel)

Surface Roughness controlling

Surface treatment (polishing)

one-way polishing

emery paper (#120, #220, #400)

0.05 μm Al_2O_3 powder (**buff**)

→ control of surface roughness

(Ra=0.05~1.20 μm)

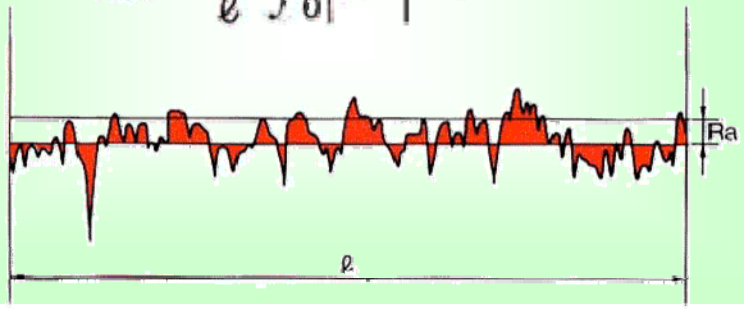
- Laser Microscope (non-contact)

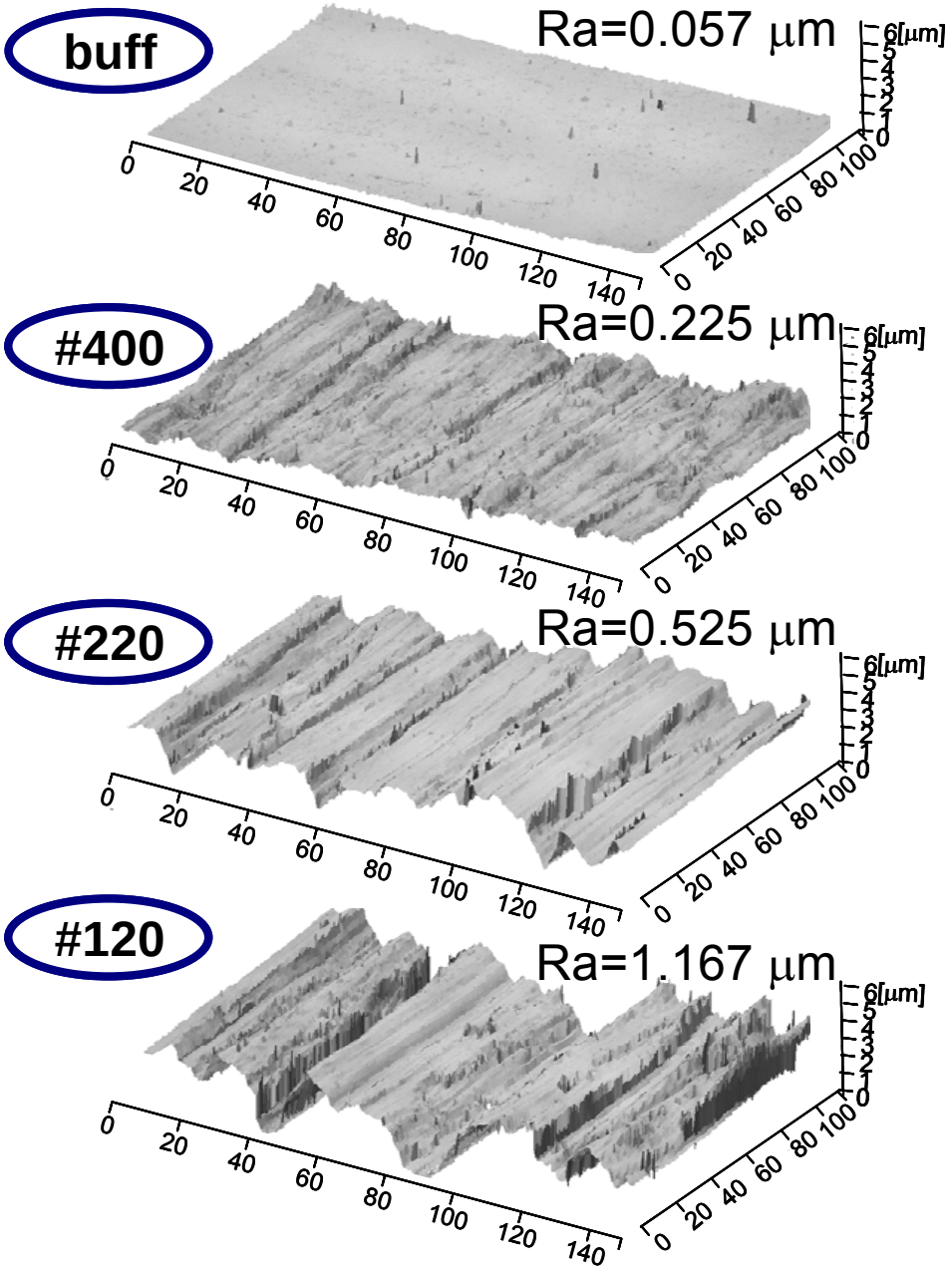
  measuring area: 150μm×112μm

- **Surface Roughness**

  (arithmetic average): **Ra**

$$Ra = \frac{1}{\ell}\int_0^{\ell} |f(x)|\,dx$$





Titania coating process

Ti substrate → **TiO_2 coating** → **autoclaving** (121°C 20min.) → **evaluation**

- buff polishing (0.05 μm Al_2O_3)
- acetone rinsing

- anodizing
- Oxidation
- Anodizing+Oxidation
- Oxidizing sol. immersion

- SEM
- XRD
- Ra
- Contact Angle
- Rest potential
- Albumin Adsorption

anodizing

solution (200mL): H_3PO_4 (~11 M)
H_2SO_4
NaOH

electrolysis W.E.: Ti
C.E.: Pt

potential: ~200 V (0.1 Vs^{-1})

Oxidation

400 °C: 2 h (in Air)
cooling in furnace

Oxidizing sol. immersion

solution (10mL): H_2O_2 + HNO_3
(H_2O_2 + $Ca(NO_3)_2$)

Temp.: 80 °C Time: ~20 min

Anodizing+Oxidation

【Anodizing】

solution(200mL): H_3PO_4
NaOH

【Oxidation】

400 °C: 2 h (capsule)
cooling in furnace

Titania coating process

- Anodizing in Aqueous Solution
- Immersion in Oxidizing Solution
- High Temperature Oxidation

Anodizing in Aqueous Solution

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Immersion in Oxidizing Solution

Immersion of Ti in the oxidizing acid solution containing H_2O_2 (more than 60 ºC).

low crystallinity **anatase** (hydrous gel)

Anodizing

Condition

【substrate】 cp Ti (JIS grade 3)

W.E. : plate (1.13 cm^2) (for in vitro)
rod (ϕ2×5 mm) (for in vivo)
(Ra 0.05~1.20 μm)

C.E. : Pt coil

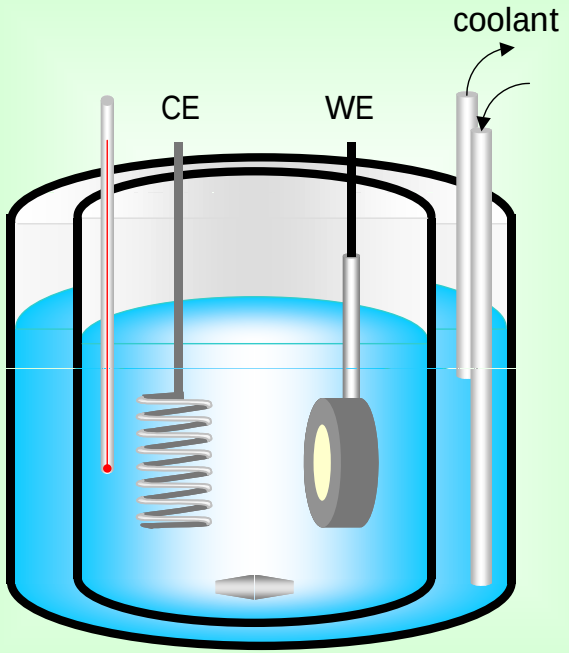
【solution】

H_2SO_4 0.1 M, 1 M
H_3PO_4 0.1~11 M
NaOH 0.1 M

【electrolysis】

potential: ~200 V
scan rate: 0.1 V s^{-1}

Apparatus



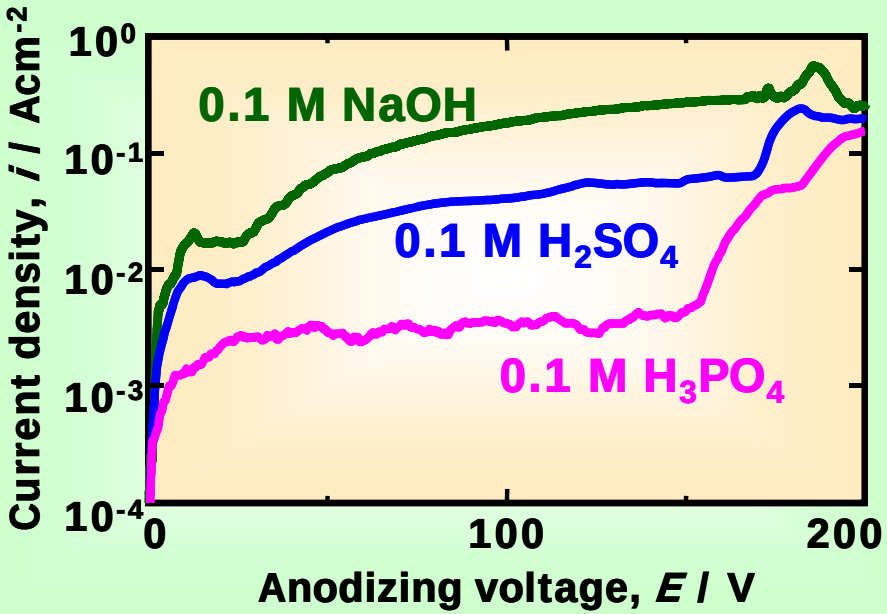
200 mL , 298 K (water bath)

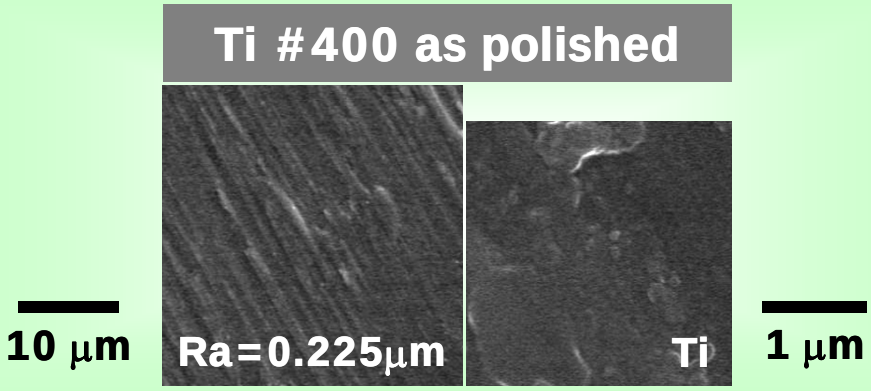
Evaluation

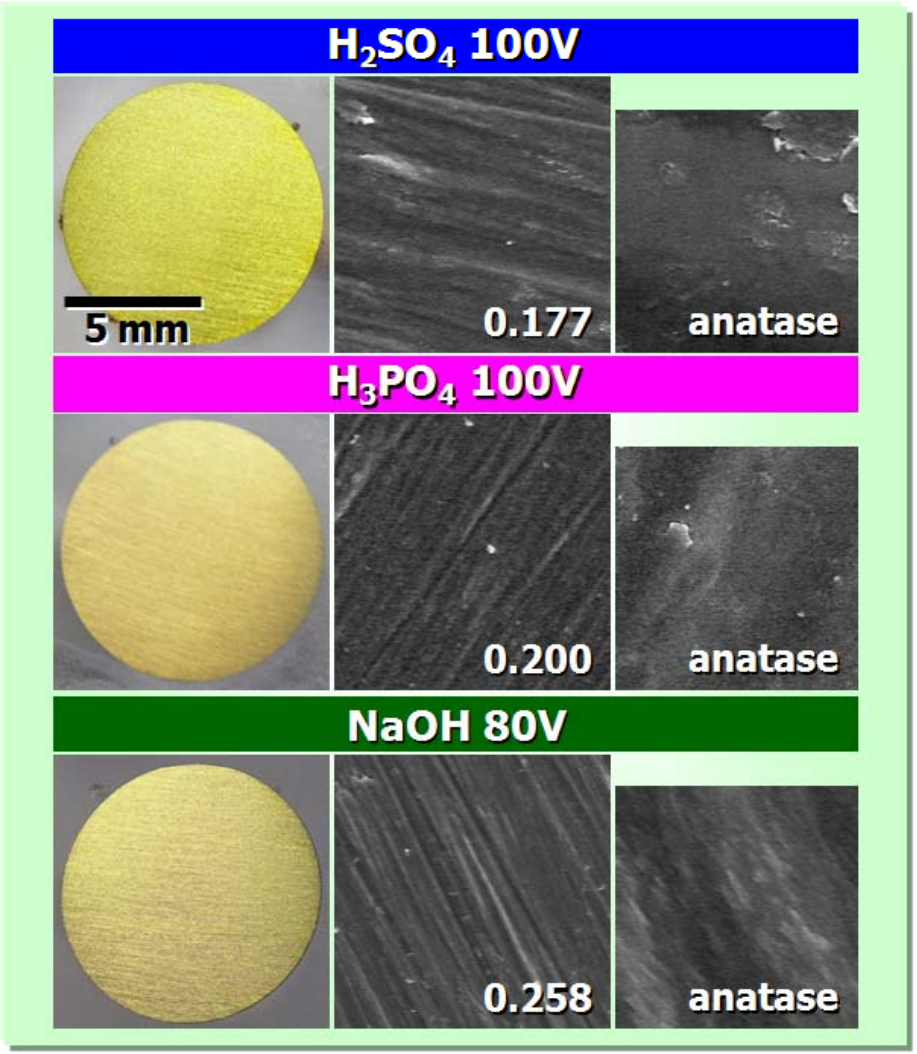
XRD, XPS
SEM
Laser Microscope

Anodizing

conc.: 0.1 M , scan rate: 0.1 V s^{-1}, polished: #400, bath temp.: 298K



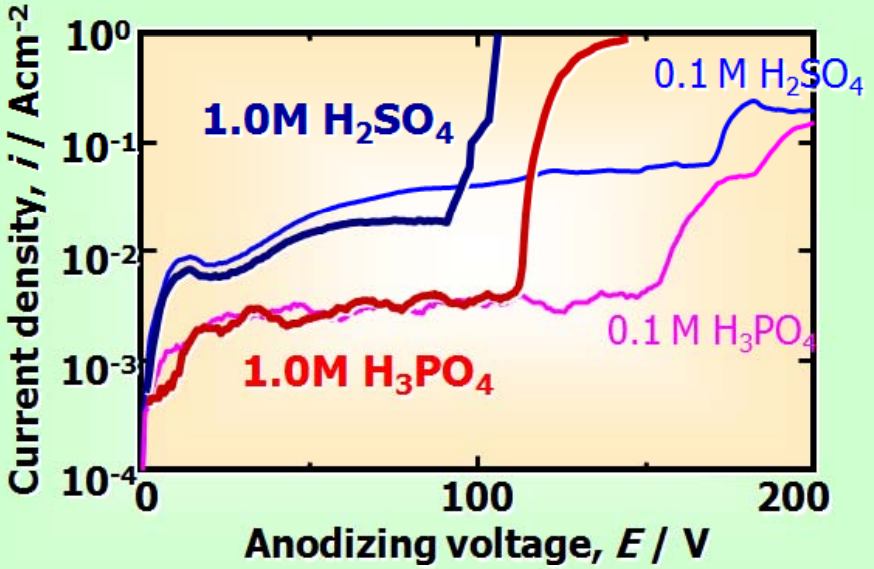


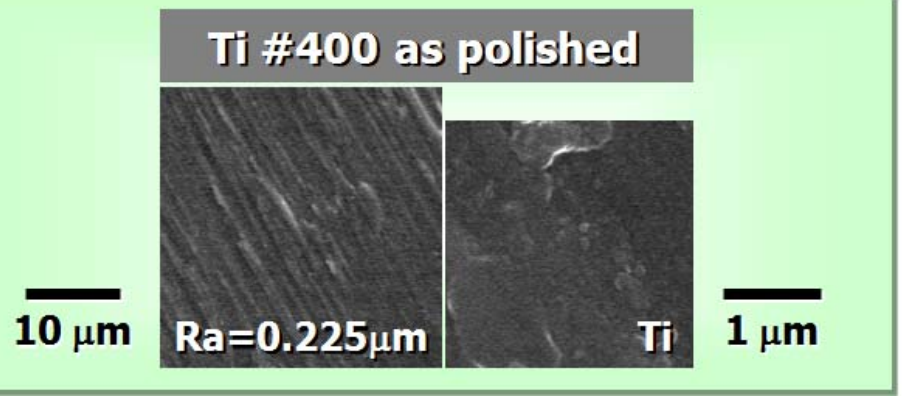


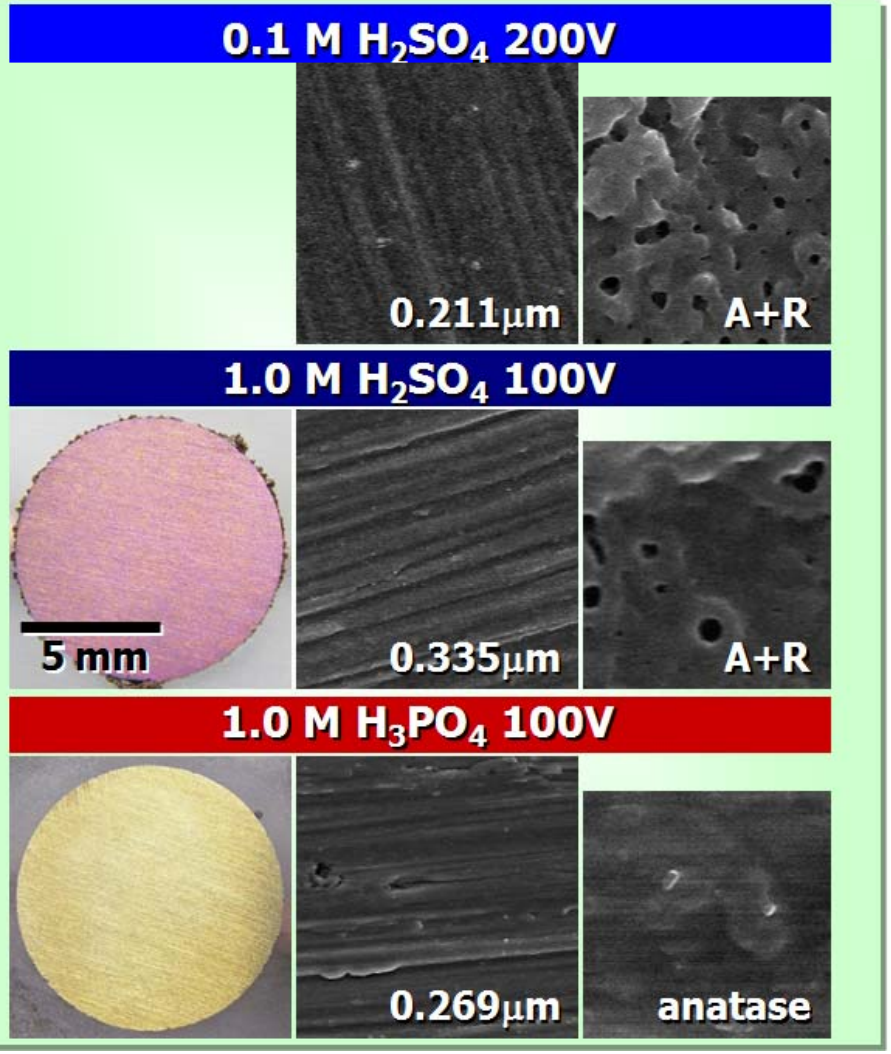
Single phase anatase films were obtained. Initial Ra was maintained.

Anodizing

scan rate: 0.1 V s^{-1}, polished: #400, bath temp.: 298K



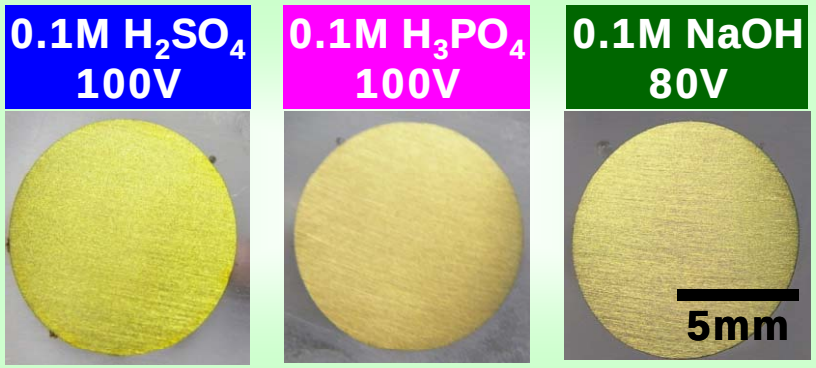


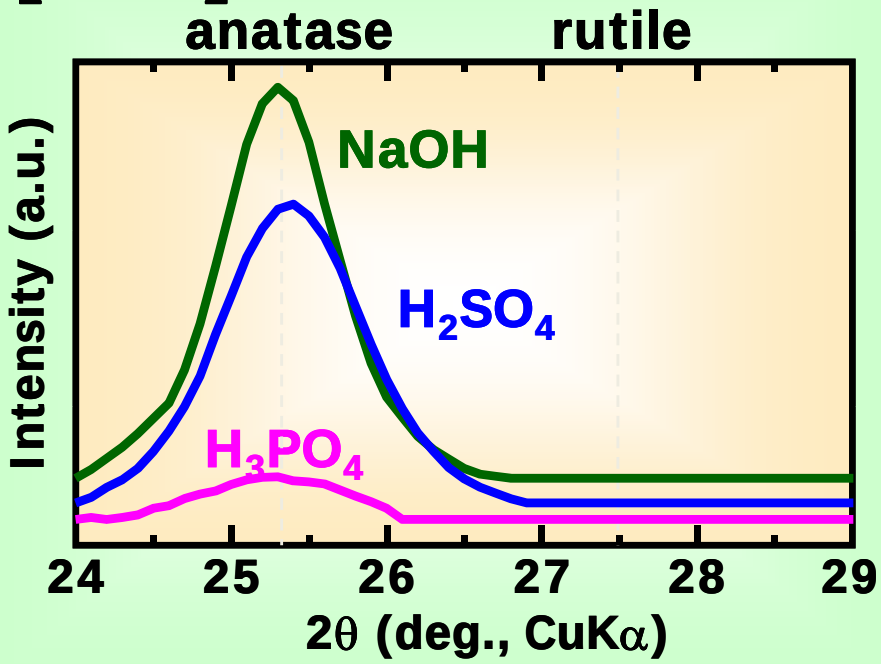


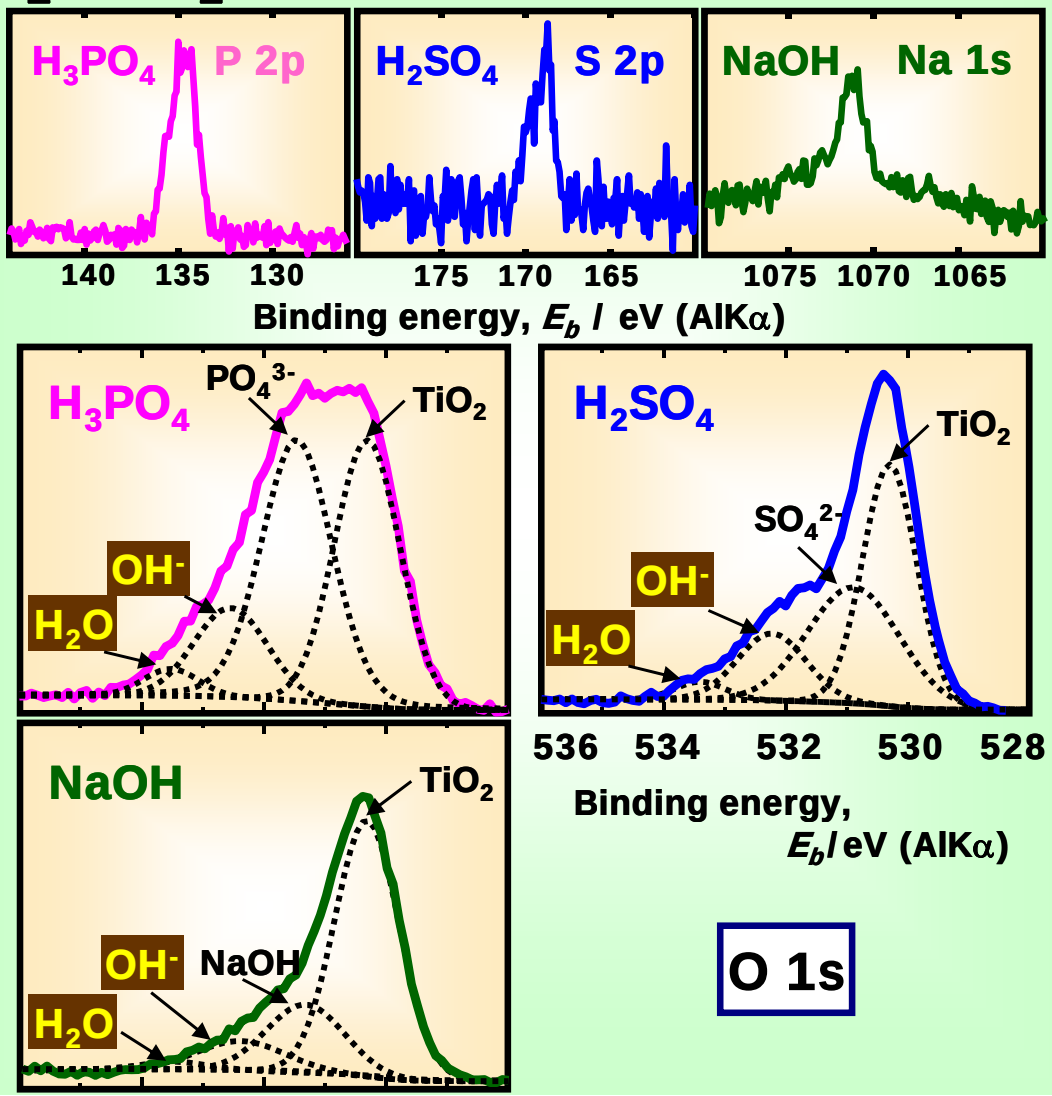
Anatase & Rutile mixed films were obtained at > sparking *E*.
And then initial Ra was not maintained.

XRD, XPS

(after autoclaving)







Anatase films had anion and cation contained the aqueous solution for anodizing.

Titania coating process

- Anodizing in Aqueous Solution
- Immersion in Oxidizing Solution
- High Temperature Oxidation

Anodizing in Aqueous Solution

Anodic electrolysis of Ti in aqueous solution

overall reaction: $Ti + 2H_2O \rightarrow TiO_2 + 4H^+ + 4e^-$

less than sparking E: **anatase,** more than: **anatase+rutile**

High Temperature Oxidation

Heating of Ti in gas phase

$Ti + O_2 \rightarrow TiO_2$ $(Ti + 1/2O_2 \rightarrow TiO)$

anatase→rutile :915 ºC
brockite→rutile :650 ºC

low temperature heating (400 ºC): **rutile** (Anatase is not obtained.)

Immersion in Oxidizing Solution

Immersion of Ti in the oxidizing acid solution containing H_2O_2 (more than 60 ºC).

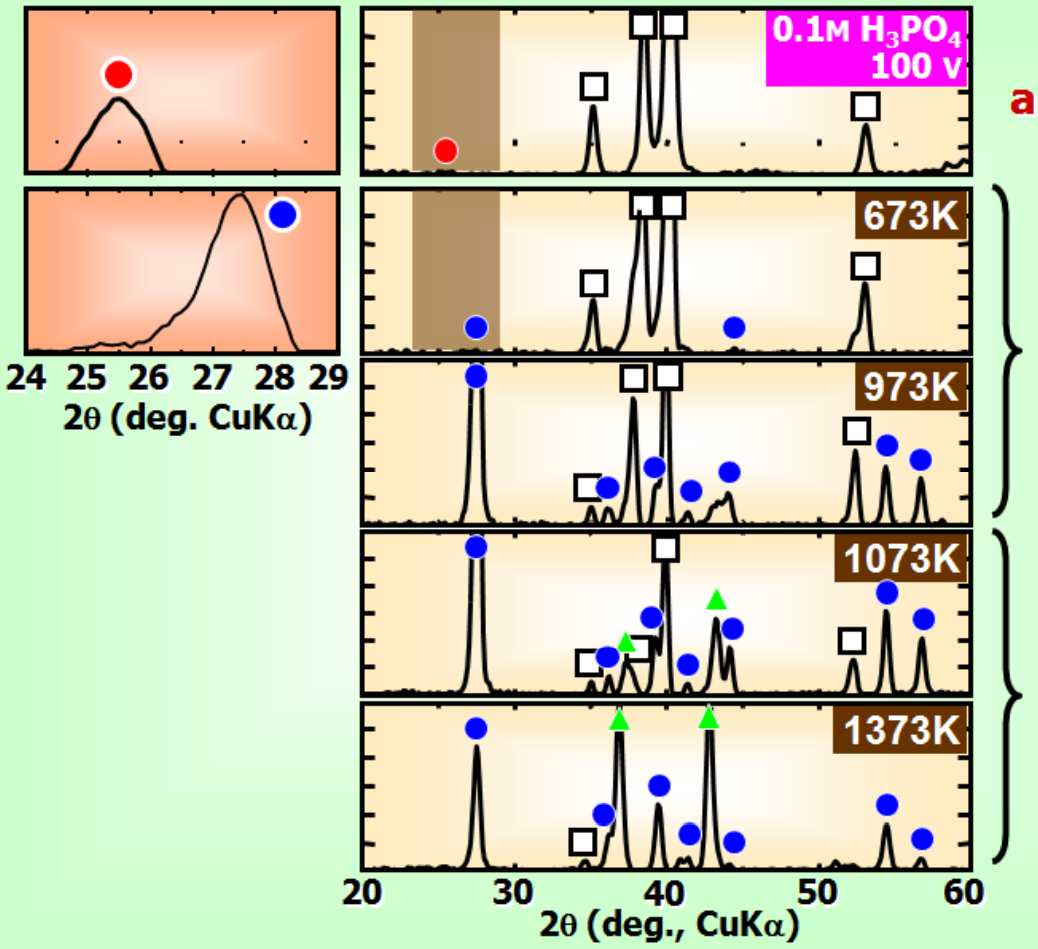
low crystallinity **anatase** (hydrous gel)

High Temp. Oxidation

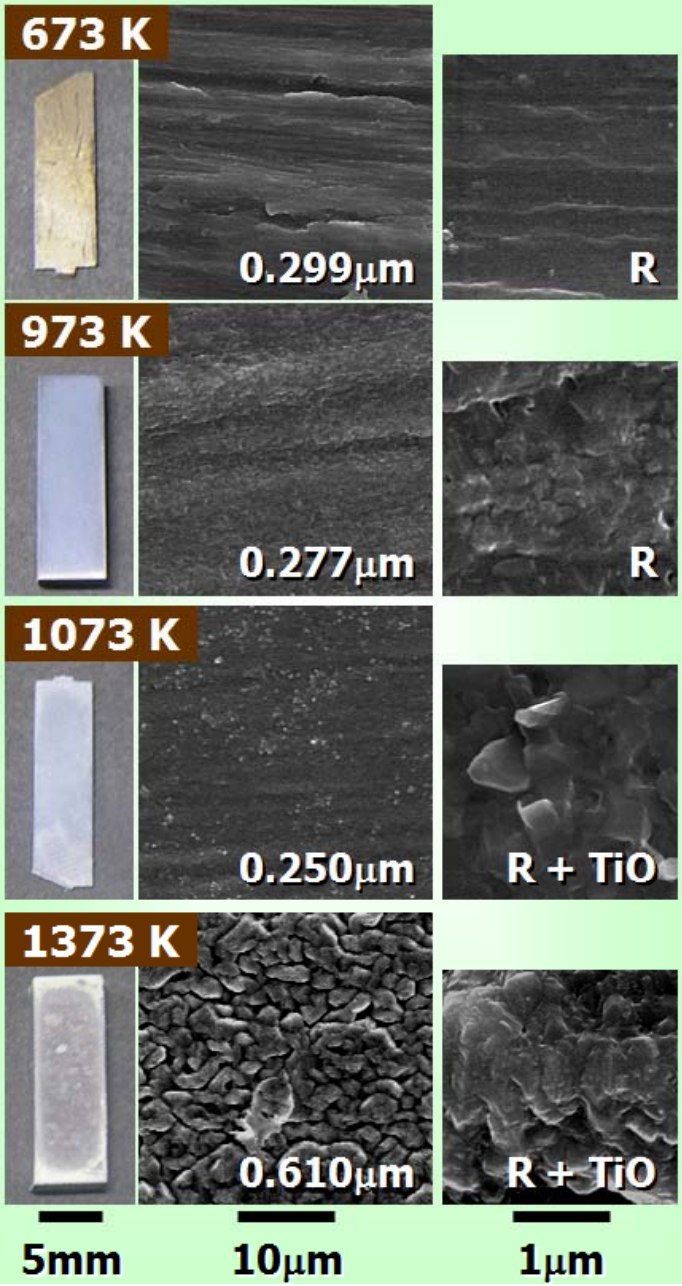
Temp.: 673~1373 K 4 K min.$^{-1}$

(2h holding, cooling in furnace)

Ar-5%H_2 Atmosphere (200 ml min.$^{-1}$)

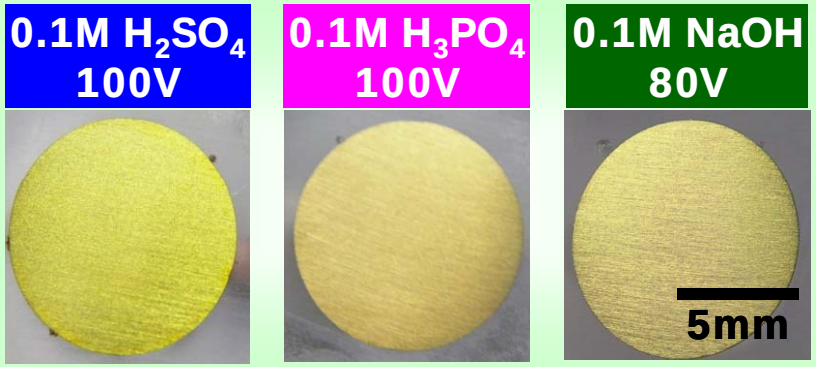


● anatase ● rutile ▲ TiO □ Ti

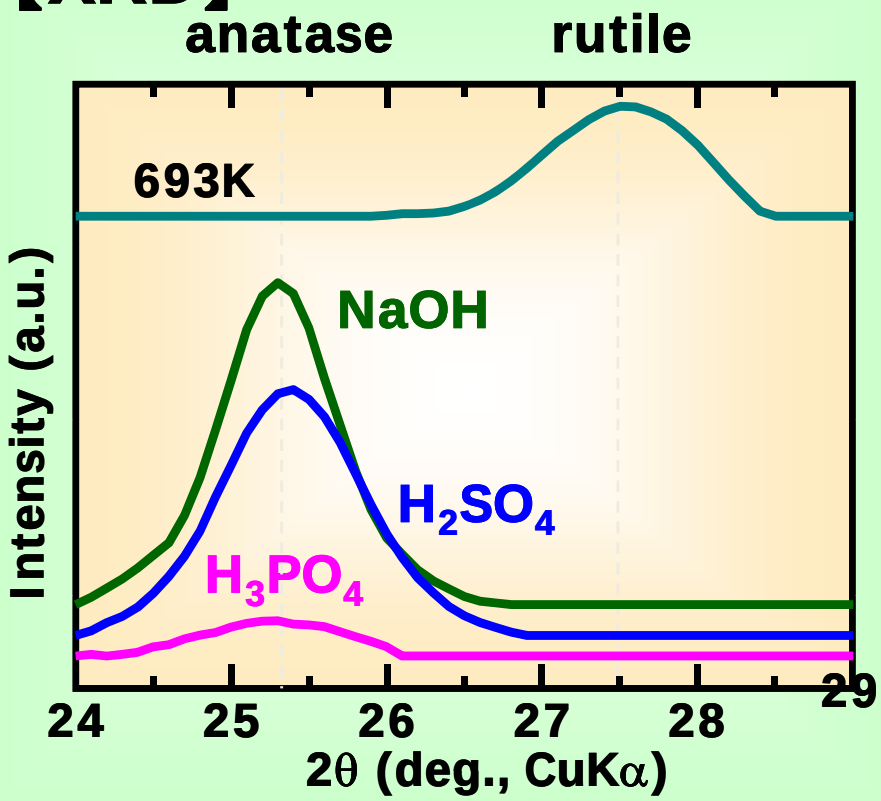


XRD, XPS

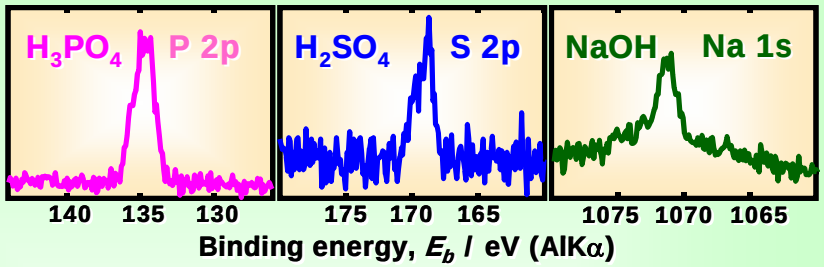
(after autoclaving)



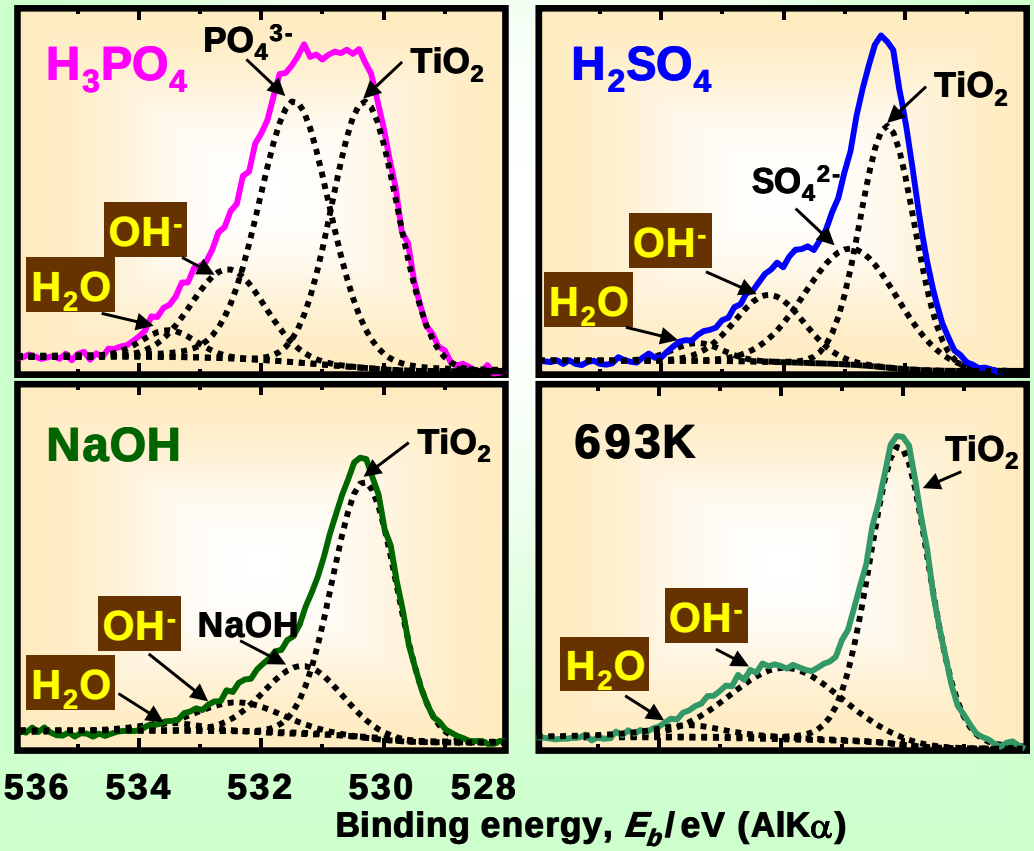
【XRD】



【XPS】



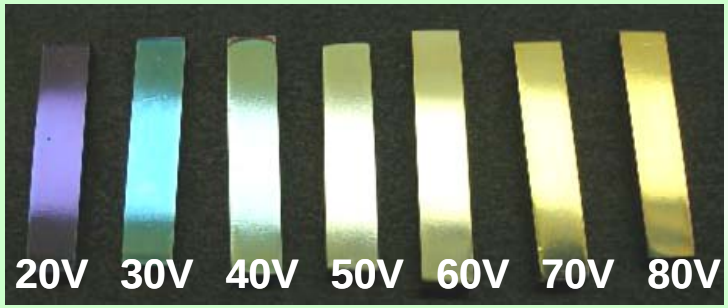
O 1s



Two-step process (anodizing + oxidation)

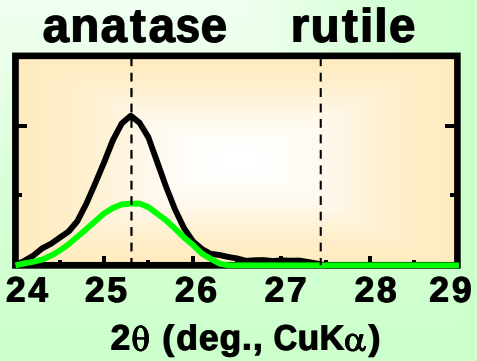
Anodizing → Oxidation （673K, 2h）

0.1M NaOH



anodizing

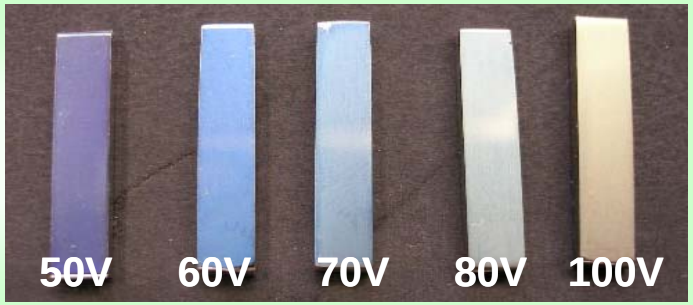
anodizing
+
oxidation

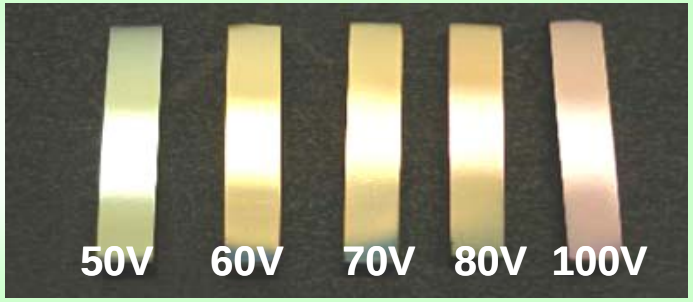


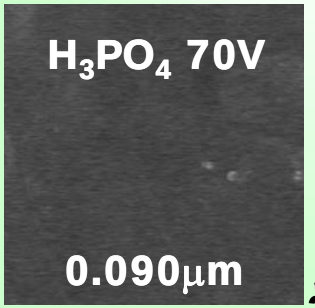
anatase growing up

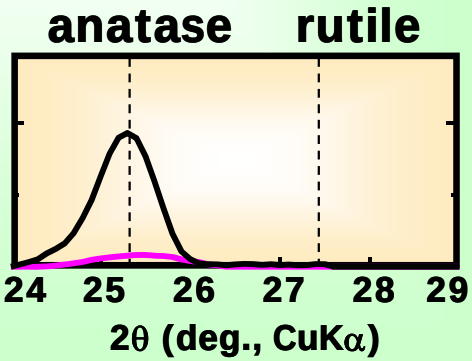
0.1M H_3PO_4

5mm









Titania coating process

- Anodizing in Aqueous Solution
- Immersion in Oxidizing Solution
- High Temperature Oxidation

Anodizing in Aqueous Solution

Anodic electrolysis of Ti in aqueous solution

overall reaction: $Ti + 2H_2O \rightarrow TiO_2 + 4H^+ + 4e^-$

less than sparking E: **anatase,** more than: **anatase+rutile**

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Heating of Ti in gas phase

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anatase→rutile :915 °C
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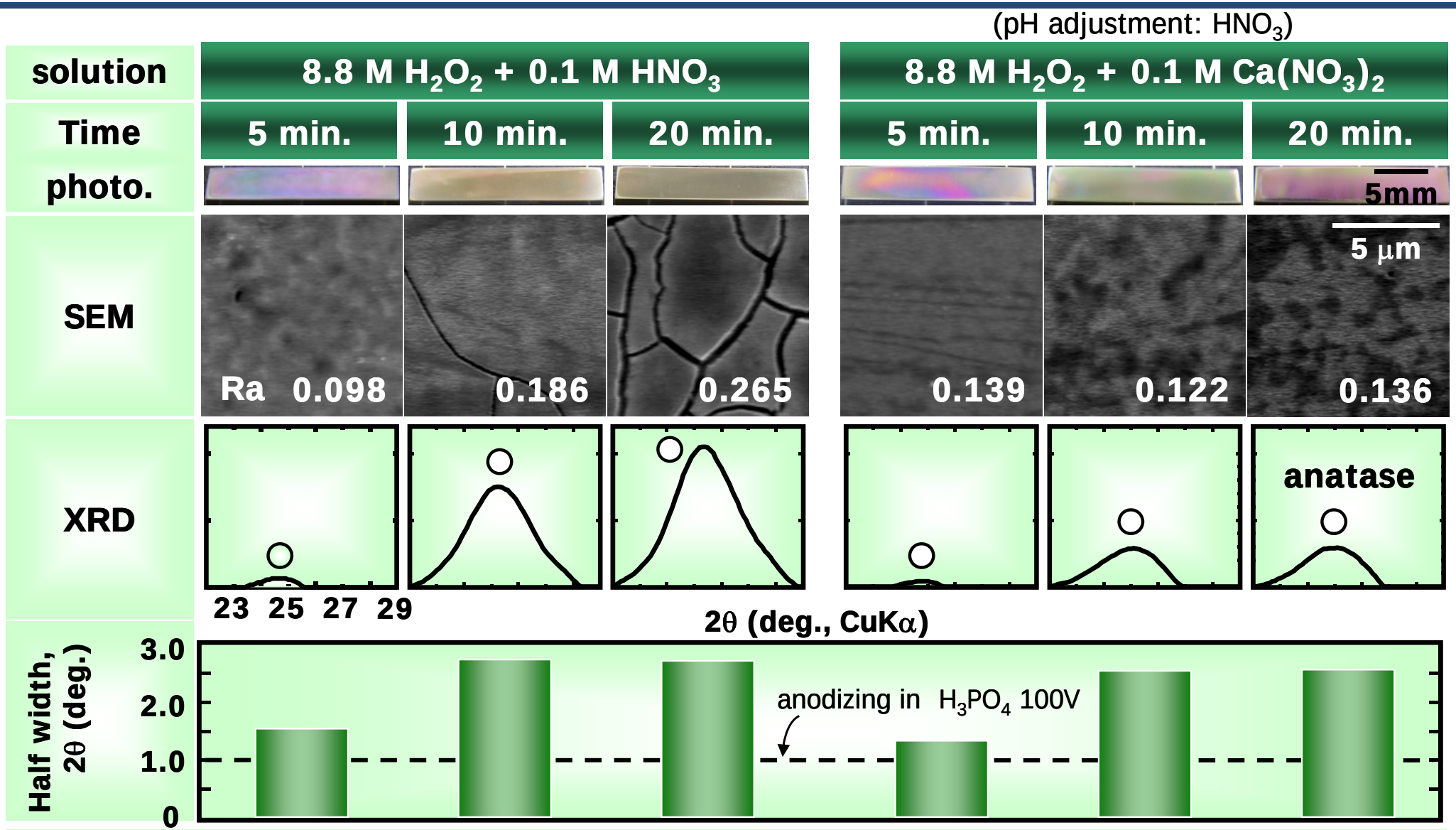
low temperature heating (400 °C): **rutile** (Anatase is not obtained.)

Immersion in Oxidizing Solution

Immersion of Ti in the oxidizing acid solution containing H_2O_2 (more than 60 °C).

low crystallinity **anatase** (hydrous gel)

Immersion in oxidizing solution



Low crystallinity **anatase** (hydrous gel) films were obtained.
The films get crack by drying.

Evaluation for Osteoconductivity

- *in vitro* (out of body)

SBF (Simulated Body Fluid) immersion

HAp nucleation and growth can be evaluated by immersing in the solution simulated inorganic components of body fluid (such as Kokubo solution, and Hank's solution).

(Organic reactions and biological reactions are ignored.)

(The equipment to keep 37°C is necessary.)

Cell culture

Osteoblasts and Osteoclasts are cultured on the samples and **cytotoxicity**, **division**, and **differentiation** are observed.

(Interactions between cells and body fluid circulation are ignored.)

(CO_2 incubator and autoclaving unit are required.)

- *in vivo*••• (in body) **Animal experiment** (implantation test)

New bone formation, bond strength, etc. are evaluated after implantation of samples into animals.

(Different results are obtained depending on used animals and evaluation methods.)

in vitro evaluation（SBF immersion）

Mater. Trans., 43(2002), 3015

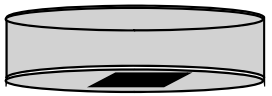
SBF, which had almost the same inorganic composition as human plasma, was used.

Inorganic ion concentrations of SBF and plasma (mM)

	Na^+	K^+	Mg^{2+}	Ca^{2+}	HCO_3^-	Cl^-	HPO_4^{2-}	SO_4^{2-}
SBF	142.0	5.0	1.5	2.5	4.2	148.0	1.0	0.5
plasma	142.0	5.0	1.5	2.5	27.0	103.0	1.0	0.5

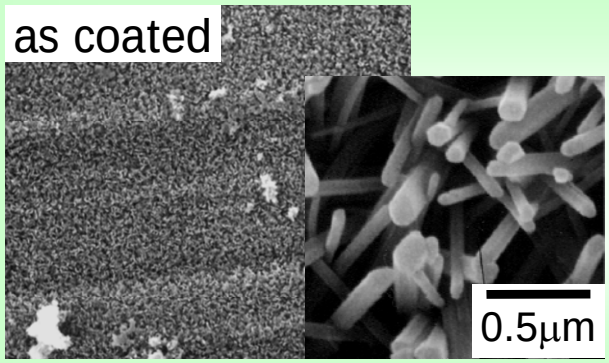
Conditions

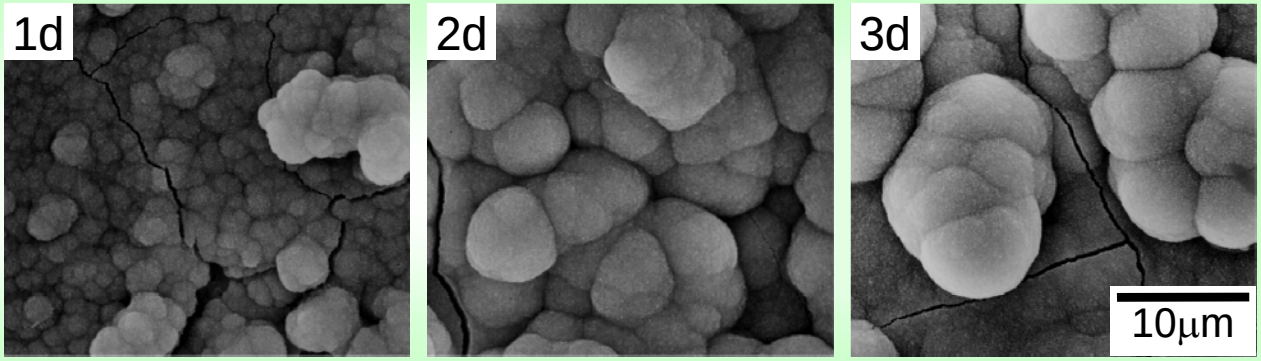
Humidity 95%
Temp. 36.5℃
Span 1h ~ 7d



Incubator

0.3mM $Ca(H_2PO_4)_2$ 0.7mM $CaCl_2$ pH8.0 150 ºC 20 min.





Bioactivity･Osteoconductivity → Nucleation and Growth of Ap

in vitro Evaluation（SBF immersion）

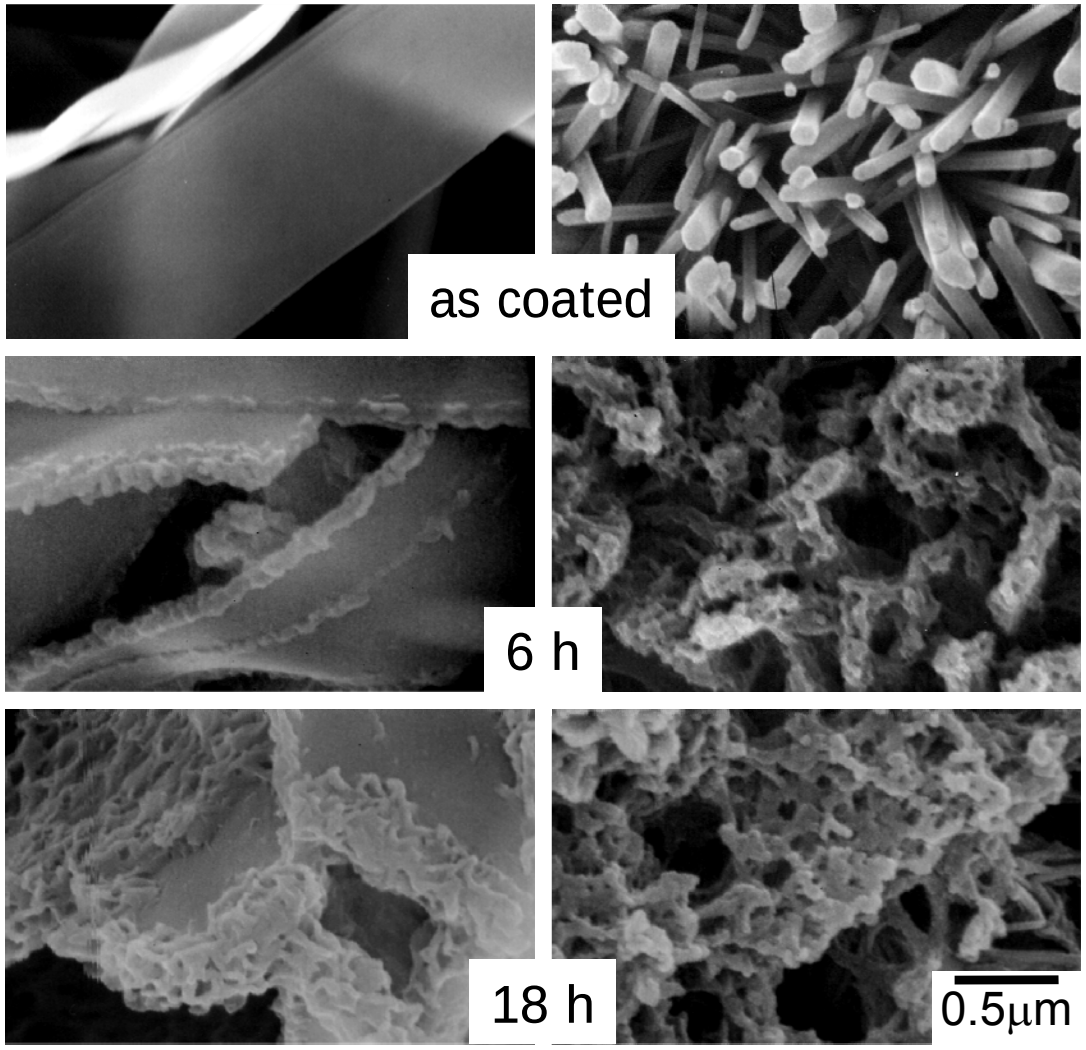
Mater. Sci.Forum, 426-432(2003), 3189

HAp coating							*in vitro* evaluation
Conditions				HAp crystal			Deposition time (to cover entire surface)
conc. /mM		pH	*T*/°C	Mor-phology	Large-ness	Density	
Ca	P						1d 2d 3d 4d 5d 6d 7~
10	6	6.0	90	Plane	Large ~40μm	Low	
	60		150				
100	6						
1	60						
1	0.6	7.0	150	Needle	Small ~2μm	High	
		8.0					
		9.0					
10	6	6.0	175	Needle	Large ~40μm	High	
		7.0	150				

Deposition time depends not on **Conditions** but on **HAp morphology.**

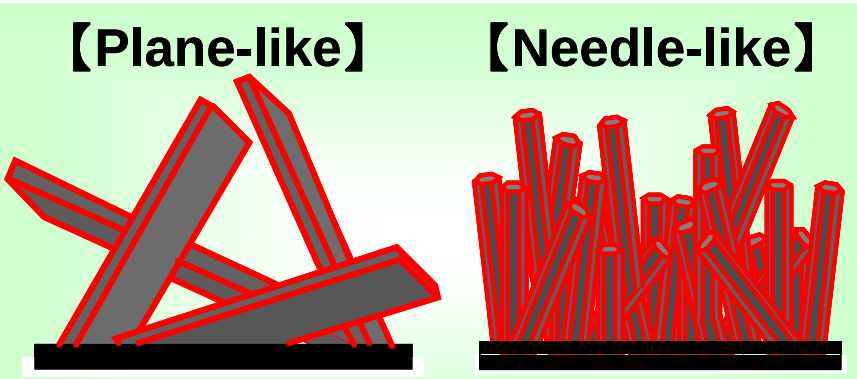
in vitro Evaluation（SBF immersion）

Mater. Sci. Forum, 426-432(2003), 3189



Active nucleation of Ap at HAp edge

Edge = Ap nucleation cyte



	Plane-like	Needle-like
Edge density	Low	High
HAp density	Low	High

The factor to influence on time needed for nucleation and growth of Ap is….

Surface morphology of HAp coatings influence on deposition rate?

in vitro evaluation (cell culture)

Osteoblast like cell **MC3T3-E1** (mouse)

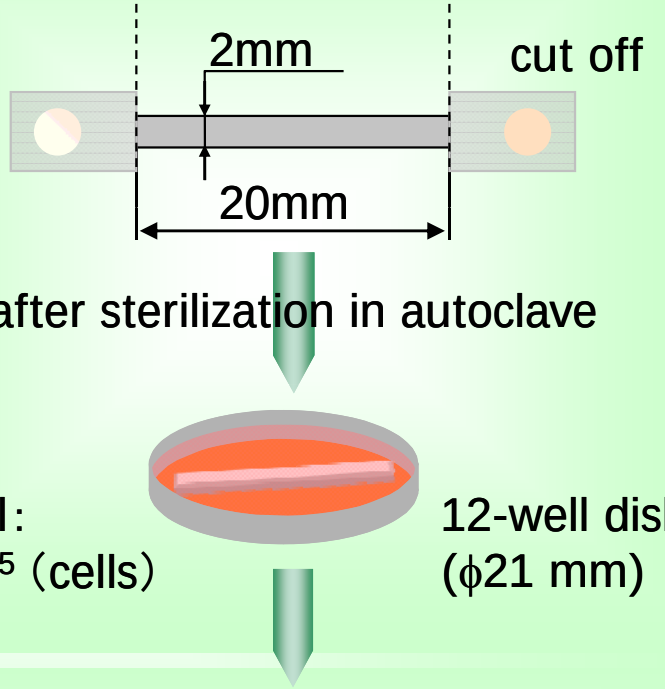
Experimental (cell culture)

【Culture media】 (1mL)

αMEM
10 vol% fetal calf serum
100 U mL^{-1} penicillin G
100 μg mL^{-1} streptomycin

【Temp.】 37 °C
【Atmos.】 Air - 5%CO_2
【Period】 1 ~ 30 d
} CO_2 incubator

【Sample】 (coated with HAp)

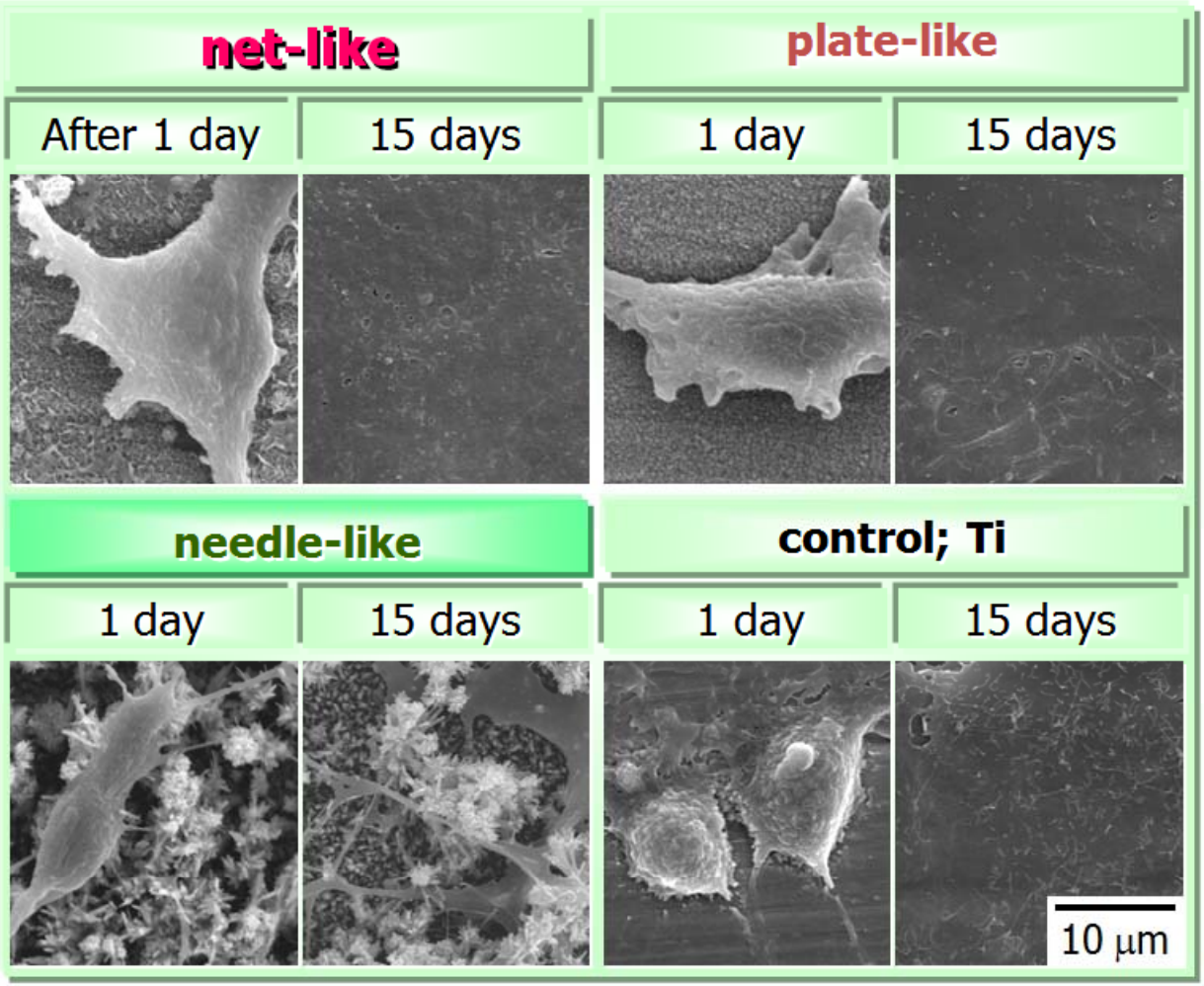


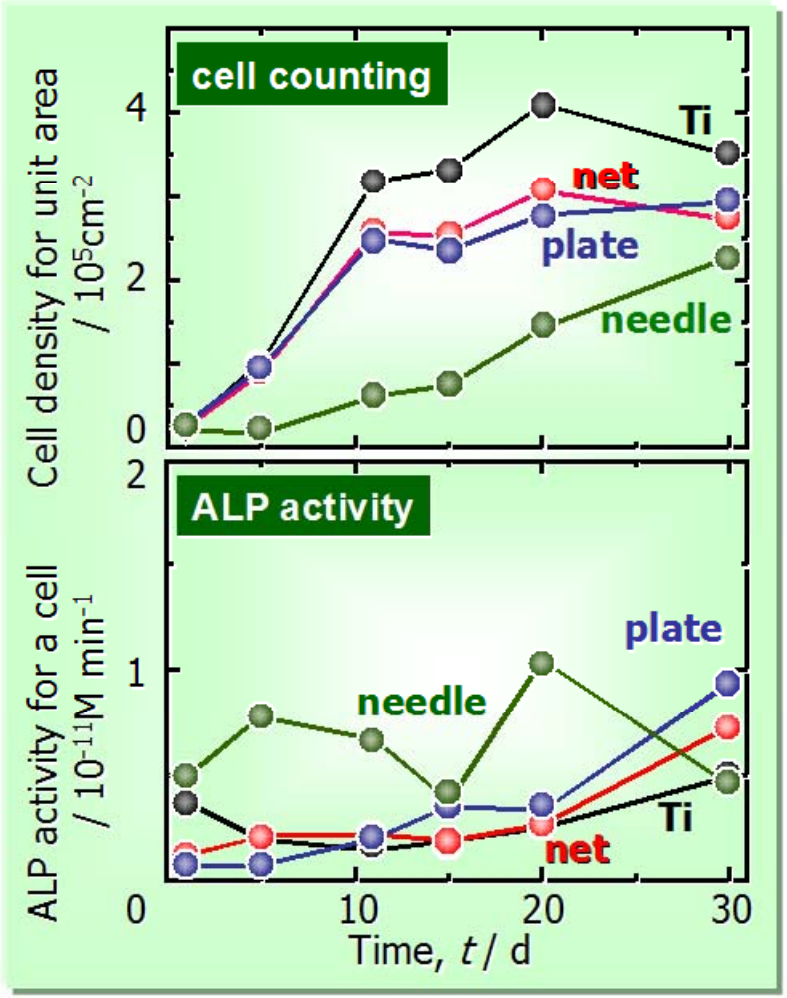
【Evaluation】

SEM observation as-proliferation
cell counting releasing by using trypsin/EDTA solution (using blood cell counting chamber)

ALP activity p-nitro phosphate (p-NP)

in vitro evaluation (cell culture)



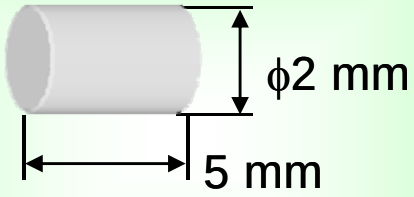


- The surface morphology is considered to be one of the most important factors in the cell growth. The order of the effectiveness for the increase in cell growth rate increased from Ti > net, plate > needle. It is not necessarily that ALP activity corresponded to the tendency of the cell growth rate.

K. Kuroda, R. Ichino, M. Okido: Mater. Sci. Forum, Vol. 539-543, p. 710-715, (2007)

in vivo evaluation

Implant (coated with HAp)



Sterilization
in steam autoclave
(121 ºC, 20 min.)

SD Rat

10-week-old, male
340-400 g
tibial metaphysis

Implantation

【Temp.】 23 ºC 【Humid.】55 ± 15 %
【Period】 2 ~ 8 weeks

Retrieving

Formation of Specimen

【Thickness】 20 μm
【Staining】 toluidine blue

Evaluation (optical microscope observation)

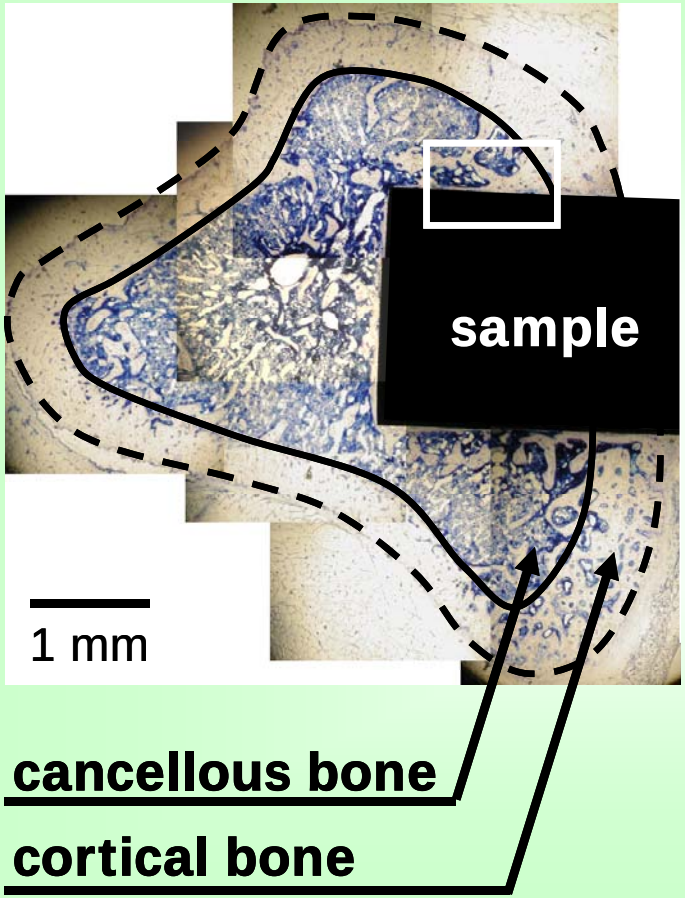
from new bone formation and tissue response

Bone-Implant contact ratio, $R_{B\text{-}I}$

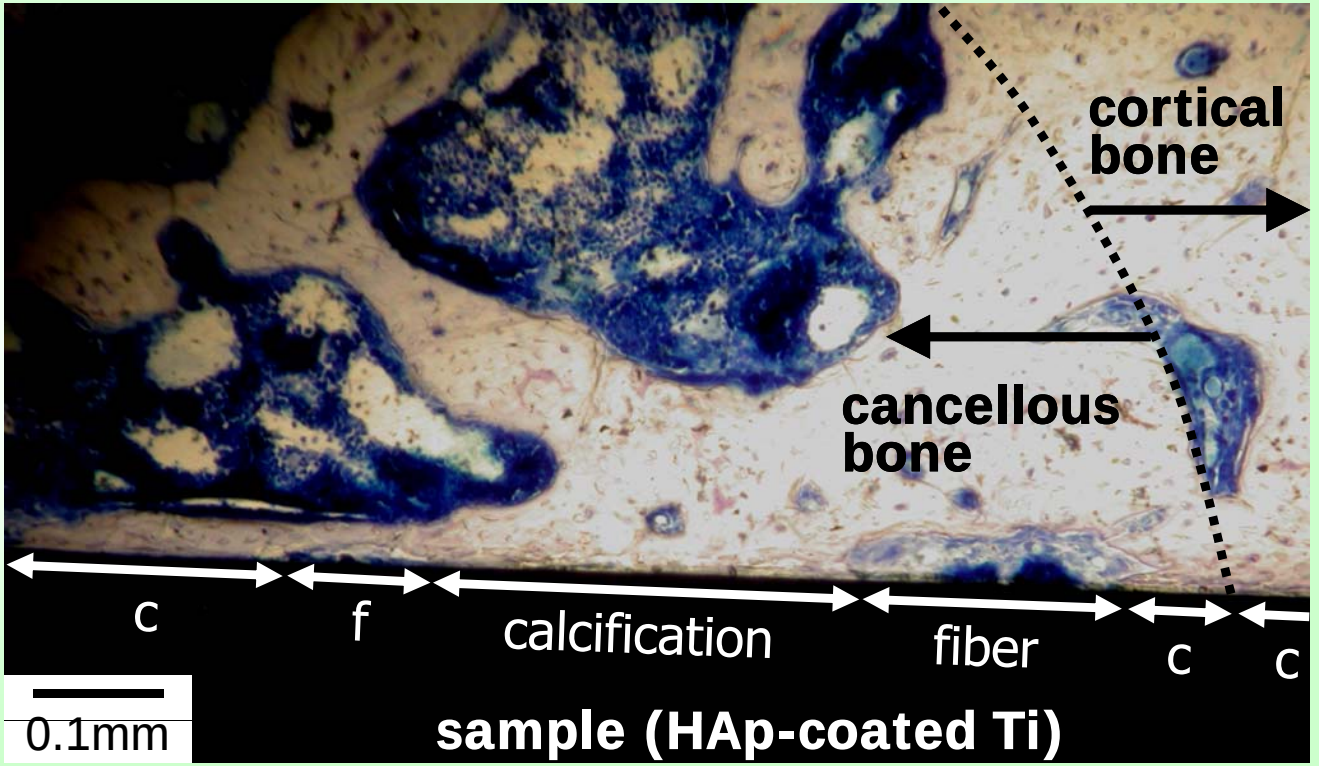
in vivo evaluation

Bone-Implant contact ratio, $R_{B\text{-}I}$

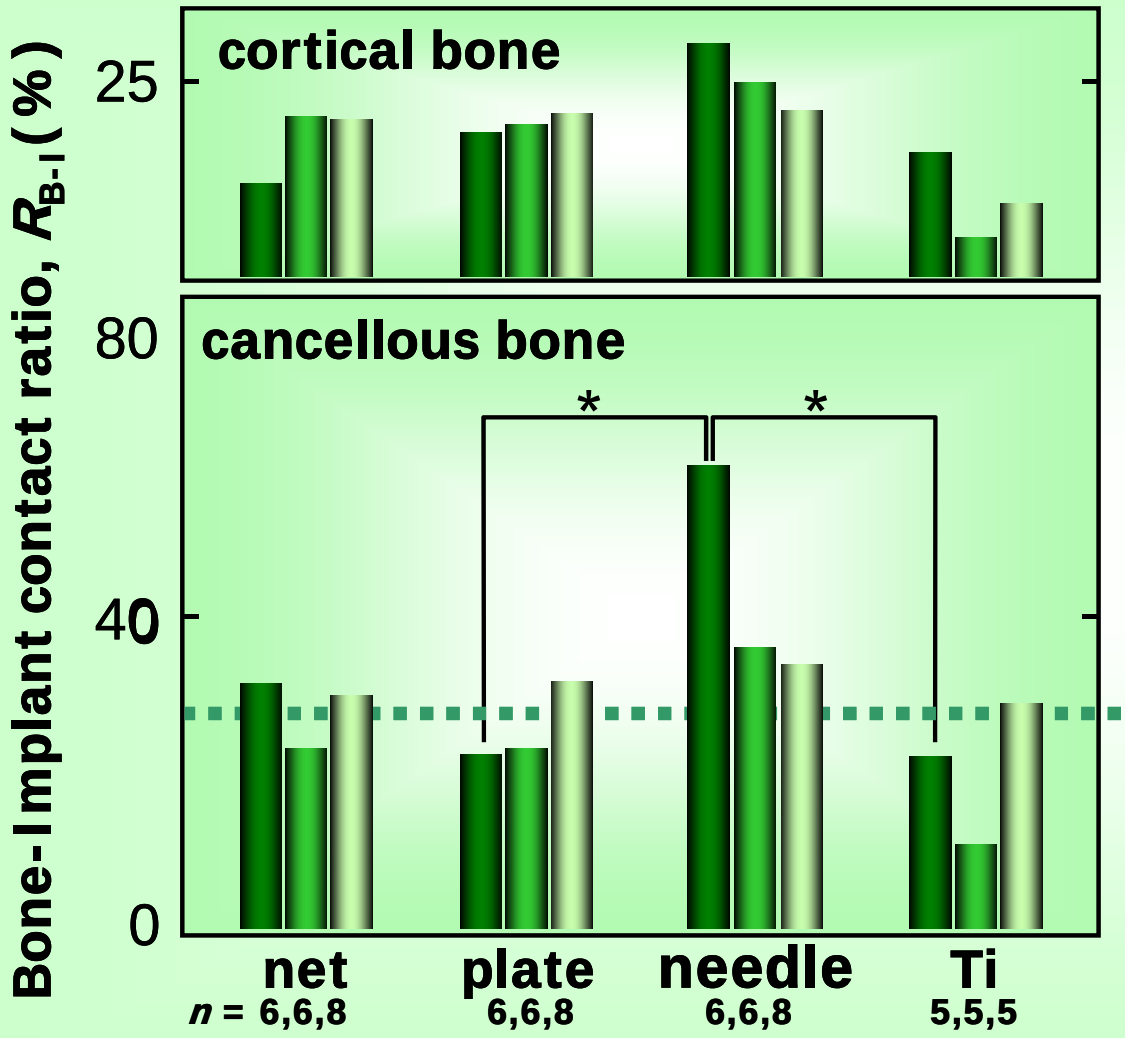
$$R_{B\text{-}I}\,(\%) = \frac{\text{total length of bone formation on implant}}{\text{total implant length}} \times 100$$



Calcification specimen



in vivo evaluation (Hap)



- **In cancellous bone, needle-like HAp samples give high osteoconductivity in early implantation stage.**
- **The effect of needle-like HAp coating lost gradually.**

30 % (natural bone)

2, 4, 8weeks

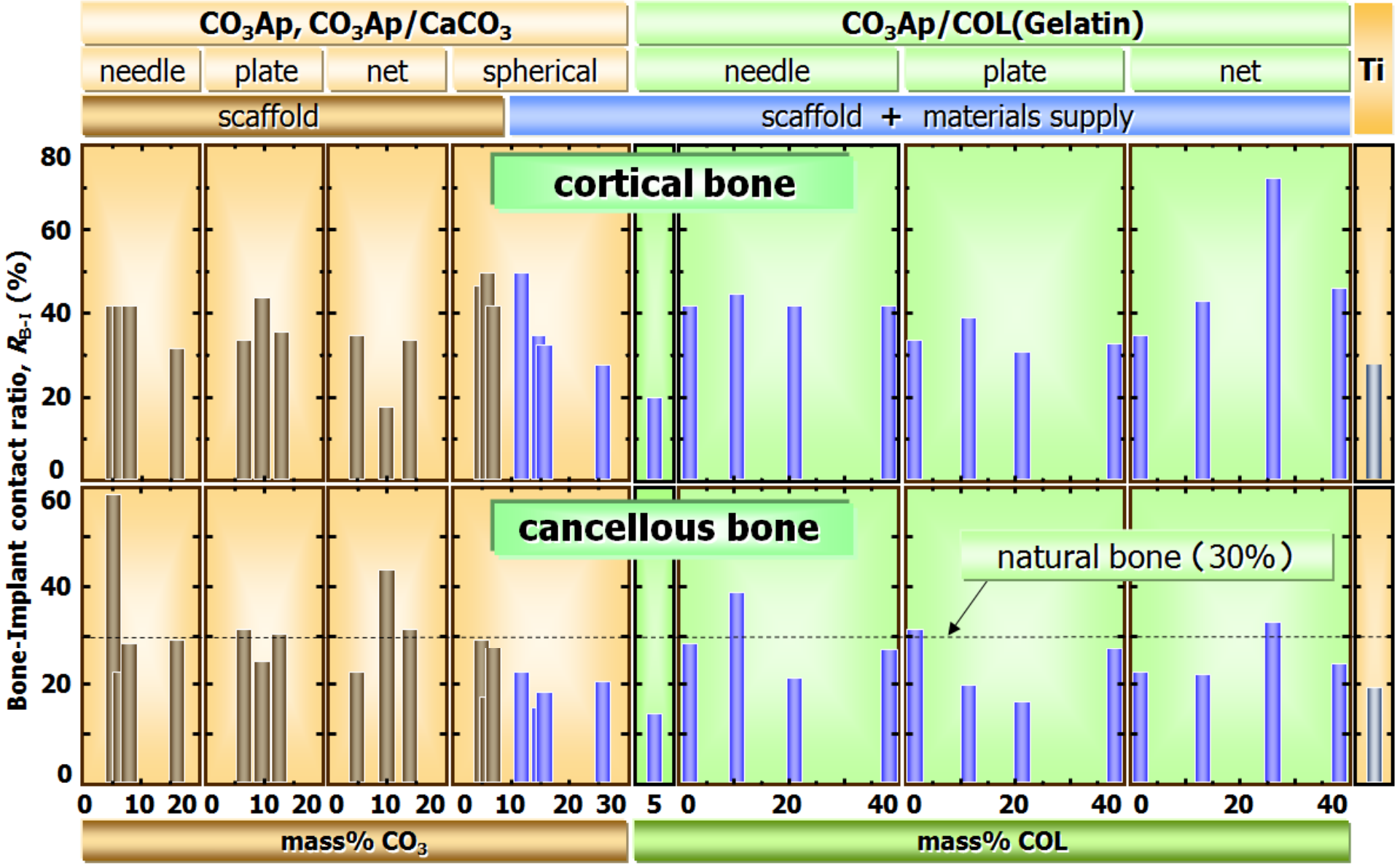
*: $p < 0.05$ (Tukey-Kramer method)

K. Kuroda, S. Nakamoto, Y. Miyashita, R. Ichino, M. Okido: Mater. Trans., Vol. 47, No. 5, p. 1931-1934,(2006)

K. Kuroda, R. Ichino, M. Okido: Mater. Sci. Forum, Vol. 539-543, p. 710-715, (2007)

in vivo evaluation (HAp)

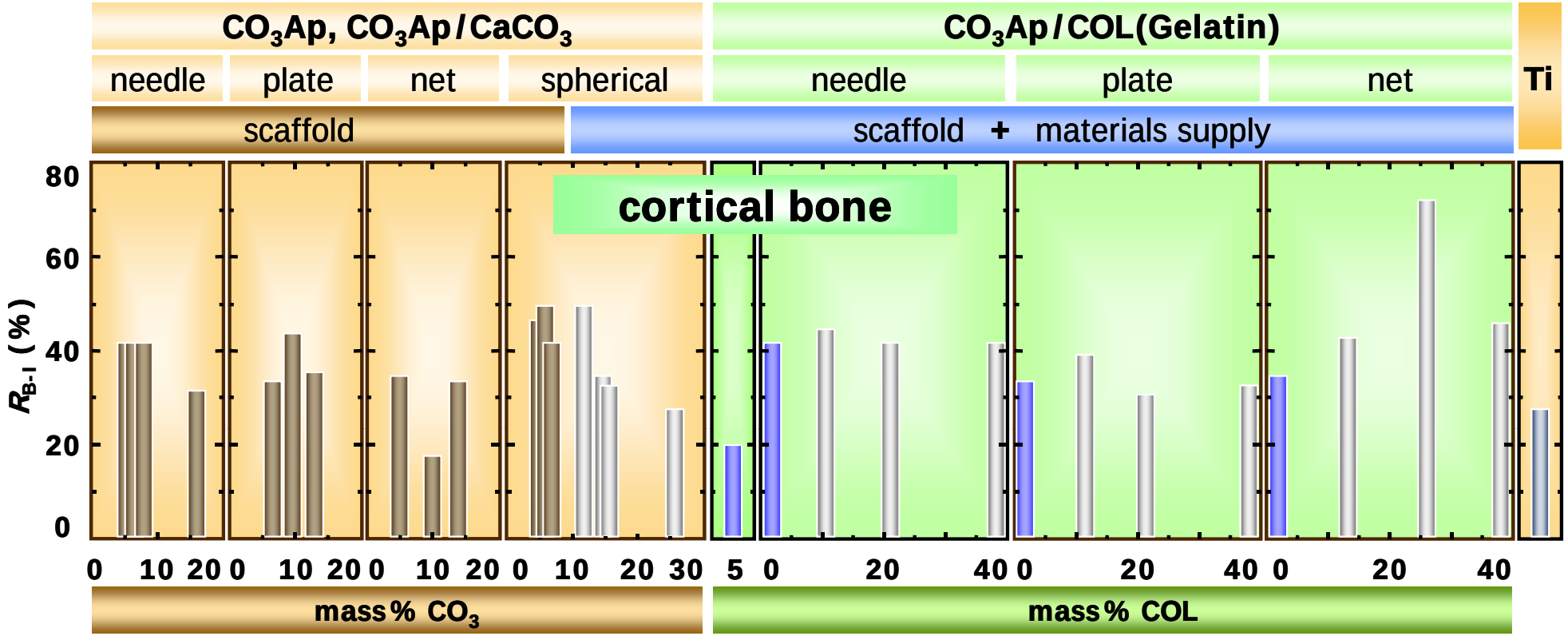
14days implantation



K. Kuroda, S. Nakamoto, Y. Miyashita, R. Ichino, M. Okido: Mater. Trans., Vol. 47, No. 5, p. 1931-1934,(2006)
K. Kuroda, M. Moriyama, R. Ichino, M. Okido, A. Seki: Mater. Trans., Vol. 49, No. 6, p. 1434-1440, (2008)
K. Kuroda, M. Moriyama, R. Ichino, M. Okido, A. Seki: Mater. Trans., Vol. 50, No. 5, p. 1190-1195, (2009)

in vivo evaluation (HAp)

14days implantation



from the viewpoint of **Scaffold**

- R_{B-I}(COL on needle) < R_{B-I}(needle) CO_3Ap enhanced osteoconductivity.
- R_{B-I}(needle, plate, net) < R_{B-I}(spherical)

K. Kuroda, S. Nakamoto, Y. Miyashita, R. Ichino, M. Okido: Mater. Trans., Vol. 47, No. 5, p. 1931-1934,(2006)
K. Kuroda, M. Moriyama, R. Ichino, M. Okido, A. Seki: Mater. Trans., Vol. 49, No. 6, p. 1434-1440, (2008)
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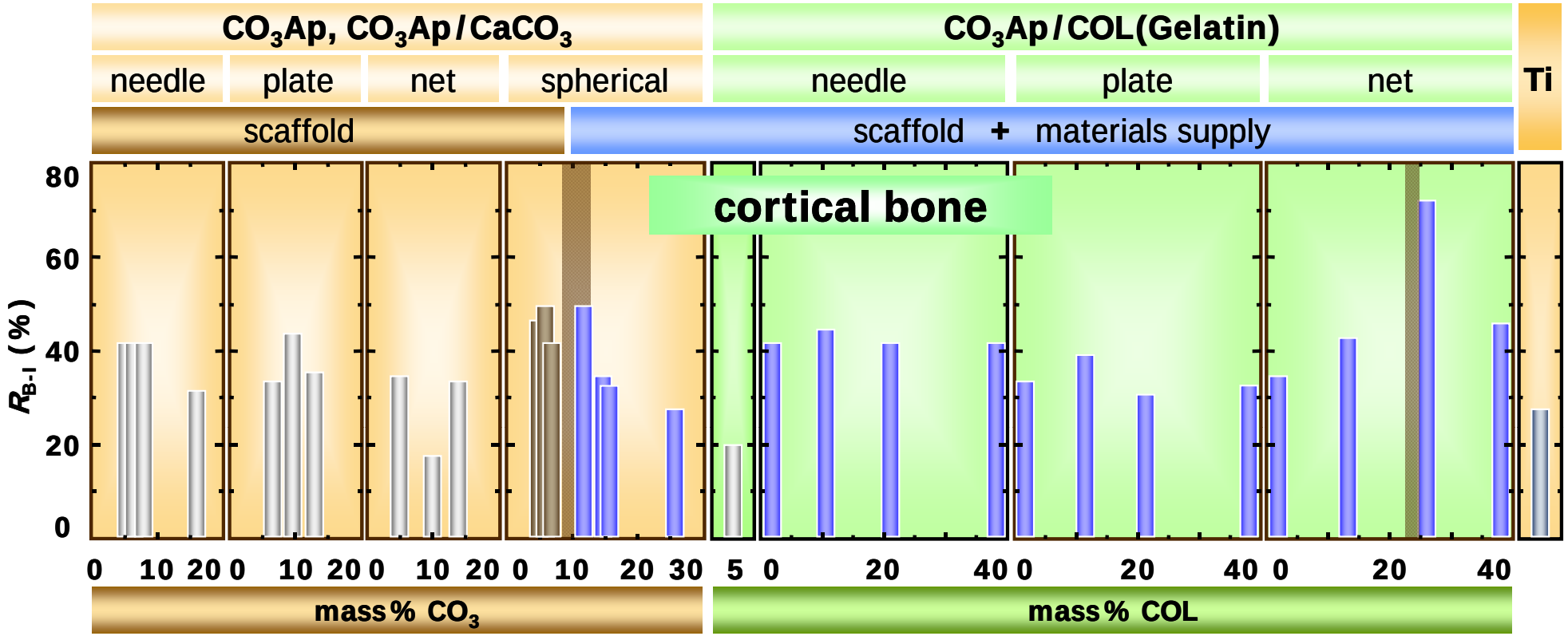
14days implantation



from the viewpoint of **scaffold + materials supply**

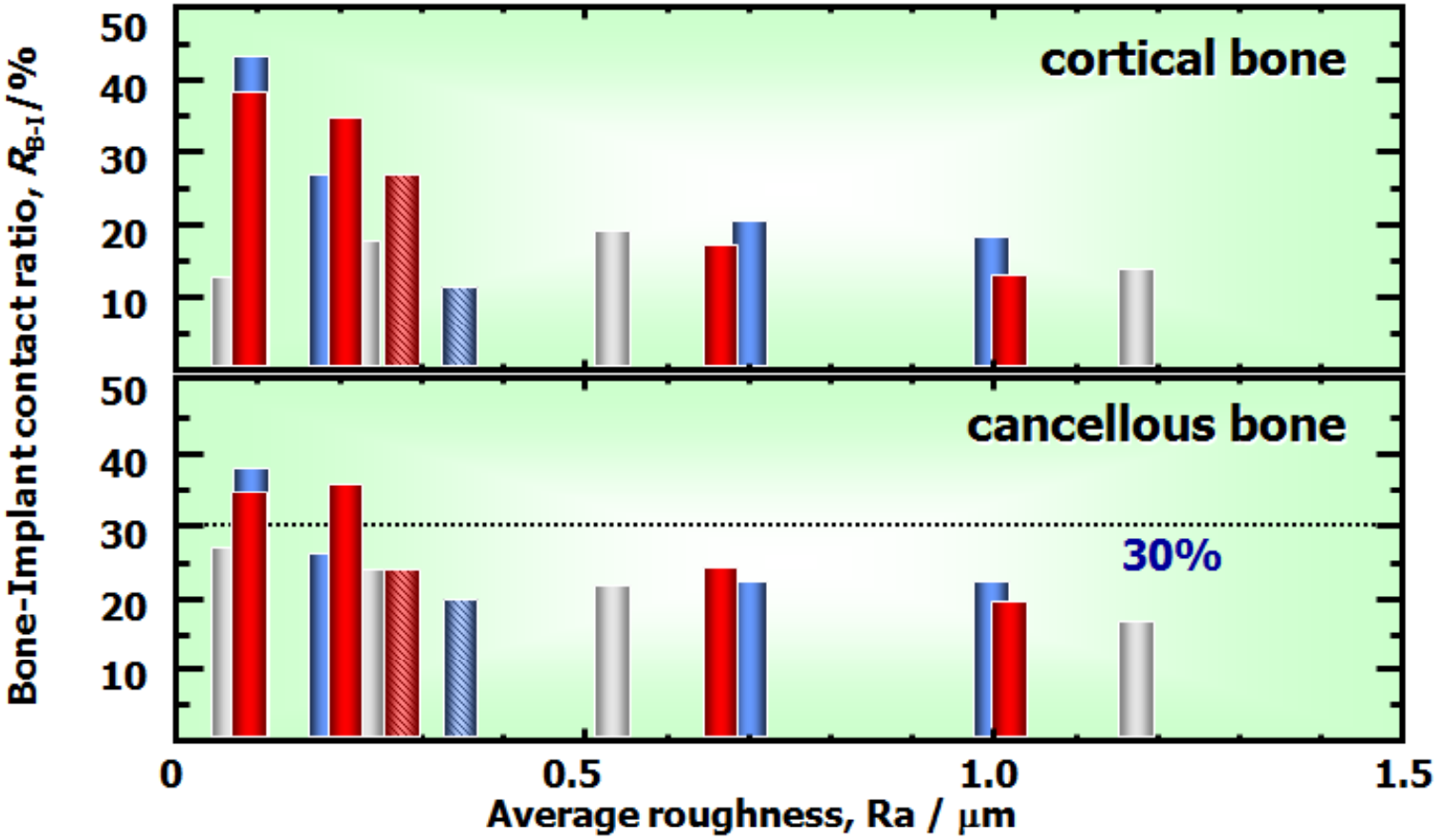
- $R_{B-I}(CO_3Ap/gelatin) = R_{B-I}(CO_3Ap) < R_{B-I}(CO_3Ap/COL)$
  Too much COL addition decreased osteoconductivity.
- $R_{B-I}(CO_3Ap) = R_{B-I}(CO_3Ap/CaCO_3)$
  Too much $CaCO_3$ addition decreased osteoconductivity.

K. Kuroda, S. Nakamoto, Y. Miyashita, R. Ichino, M. Okido: Mater. Trans., Vol. 47, No. 5, p. 1931-1934,(2006)
K. Kuroda, M. Moriyama, R. Ichino, M. Okido, A. Seki: Mater. Trans., Vol. 49, No. 6, p. 1434-1440, (2008)
K. Kuroda, M. Moriyama, R. Ichino, M. Okido, A. Seki: Mater. Trans., Vol. 50, No. 5, p. 1190-1195, (2009)

in vivo evaluation (anodizing)

- **Anodizing in H_3PO_4 and H_2SO_4**
- **14 days implantation**

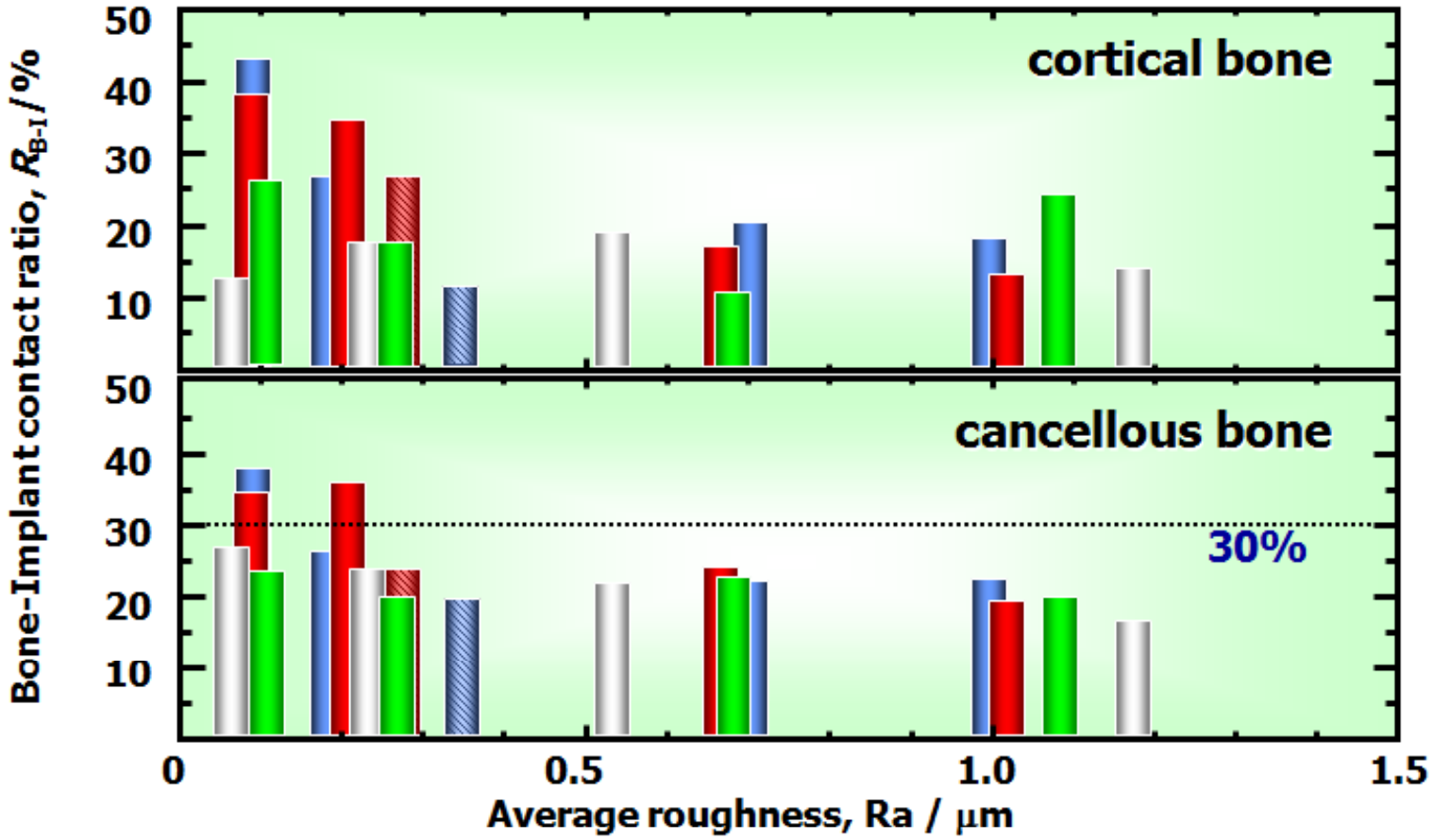
0.1M H_2SO_4
as polished
0.1M H_3PO_4



- TiO_2 coating enhanced the osteoconductivity.
- Surface roughness of TiO_2 coating influenced on the osteoconductivity.
- R_{B-I} (TiO_2 in H_2SO_4) = R_{B-I} (TiO_2 in H_3PO_4)

in vivo evaluation (anodizing)

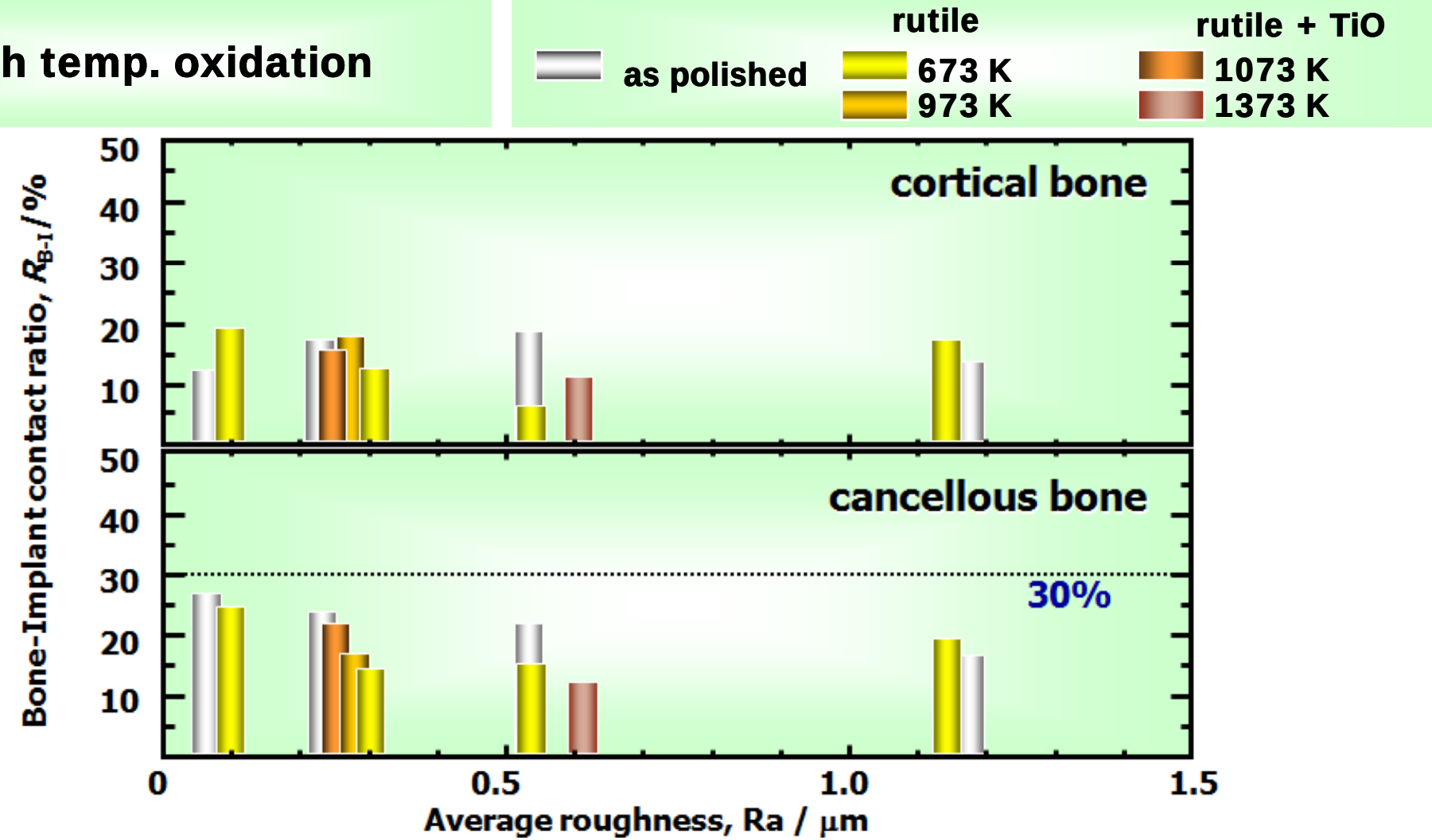
- **Anodizing in H_3PO_4, H_2SO_4 and NaOH.**



- The osteoconductivity of TiO_2 coating anodized in NaOHaq depended on the surface roughness.
- R_{B-I} (TiO_2 in H_2SO_4) = R_{B-I} (TiO_2 in H_3PO_4) > R_{B-I} (TiO_2 in H_3PO_4)

in vivo evaluation (oxidation)

- **High temp. oxidation**

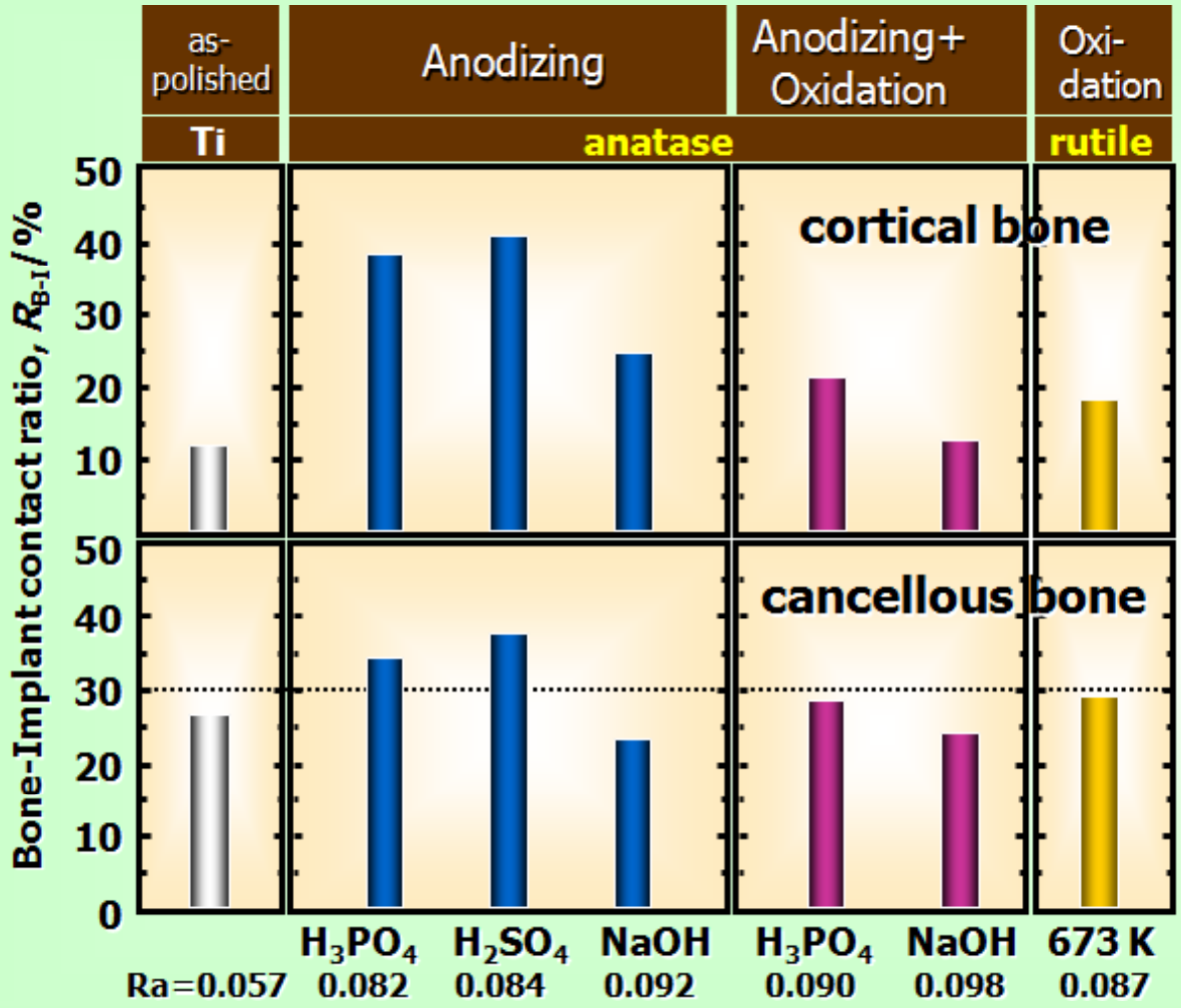


- TiO_2 coating made by high temp. oxidation did NOT improve the osteoconductivity.
- The effect of the surface roughness of TiO_2 coating was not clear.

in vivo evaluation (TiO2)

Anodizing	**0.1 M H_3PO_4(100 V), 0.1 M H_2SO_4 (100 V), 0.1 M NaOH (80 V)**
Anodizing+Oxidation	**0.1 M H_3PO_4(100 V), 0.1 M NaOH (80 V) + 673 K (2 h) in vac.**
Oxidation	**673 K (2 h) in Ar-5%H_2**

（120nm thickness）



R_{B-I}(Anodizing)＞
R_{B-I}(Anodizing+Oxidation)

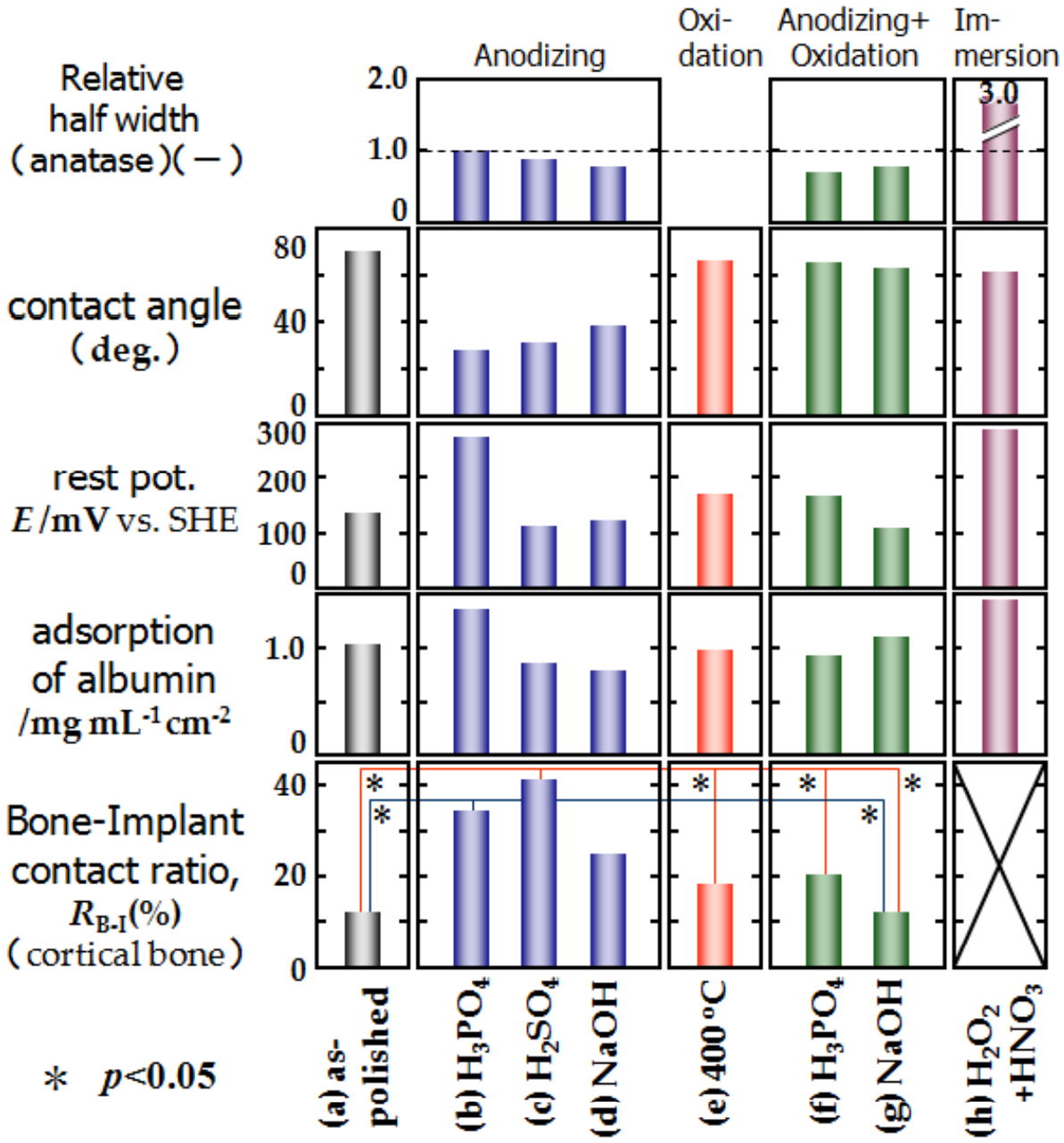
even if same anatase

R_{B-I}(Anodizing+Oxidation)＝
R_{B-I}(oxidation)

Oxidation decreased R_{B-I}.
(anatase&rutile)

TiO_2 coating formed in the hydro-process enhanced the osteoconductivity.

Evaluation (from the viewpoint of engineering)



【Relative half width】
from XRD chart (0.1M H_3PO_4 100V: 1)

【contact angle】
2 μL water droplet (RT)

【rest potential】
in PBS(-) at 37 °C pH 7.4 (dark)

【adsorption of albumin】
in PBS(-) at 37 °C pH 7.4. MicroBCA.

(a) as-polished
(buffing, 0.05μm Al_2O_3)

(b) 0.1M H_3PO_4, 100V

(c) 0.1M H_2SO_4, 100V — Anodizing

(d) 0.1M NaOH, 80V

(e) Air, 400°C, 2h — Oxidation

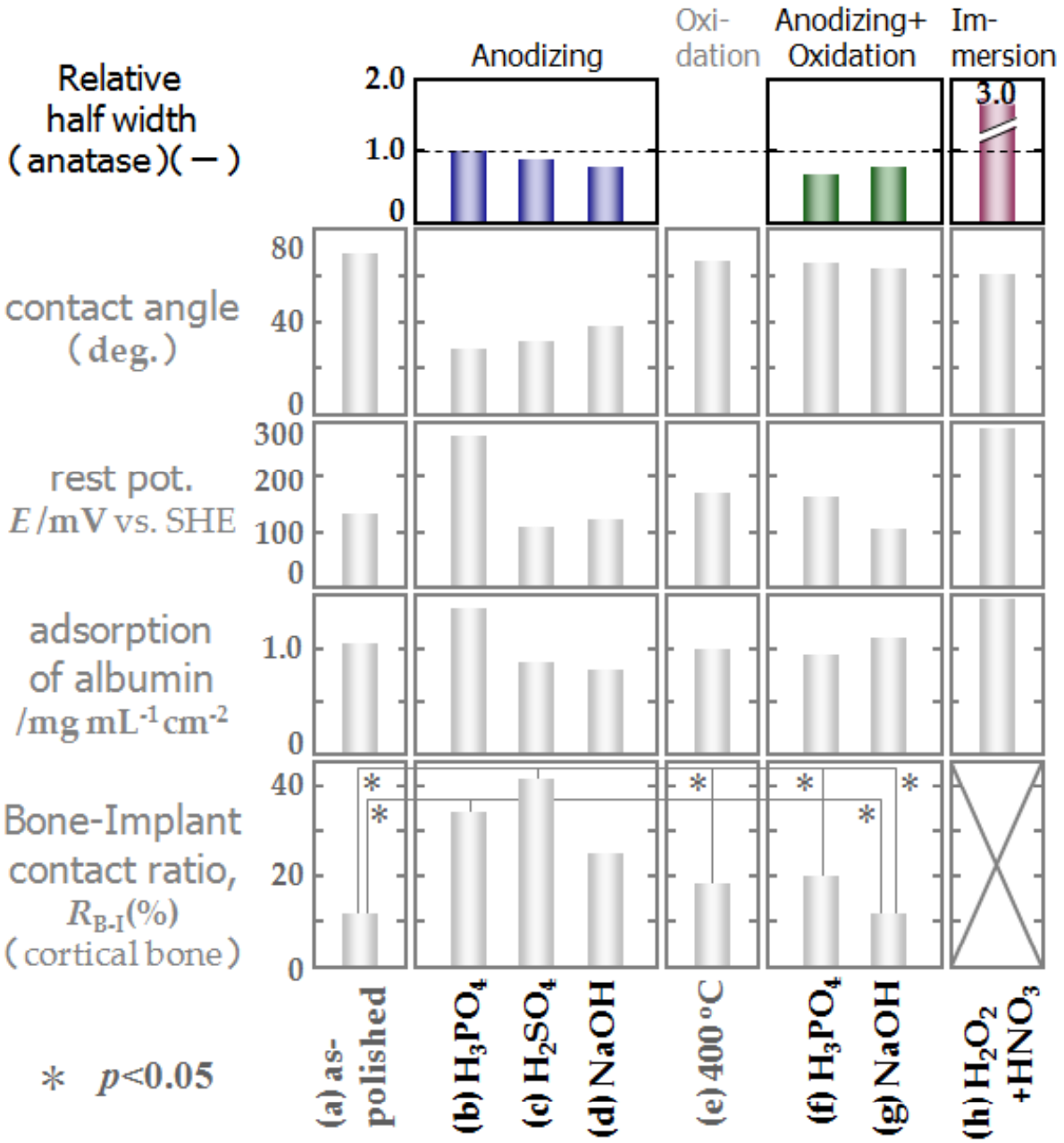
Anodizing+Oxidation

(f) 0.1M H_3PO_4, 70V+Vac., 400°C, 2h

(g) 0.1M NaOH, 50V+Vac., 400°C, 2h

(h) 8.8M H_2O_2+0.1M HNO_3
(80°C, 20min) — Immersion

Evaluation (crystallinity)



- Anodized TiO_2 films have low crystallinity. (depend on the solution)
- Oxidation process increase the TiO_2 crystallinity.
- TiO_2 gel films made by the immersion in the oxidizing solution have low.

(a) as-polished (buffing, 0.05μm Al_2O_3)

(b) 0.1M H_3PO_4, 100V

(c) 0.1M H_2SO_4, 100V — Anodizing

(d) 0.1M NaOH, 80V

(e) Air, 400°C, 2h — Oxidation

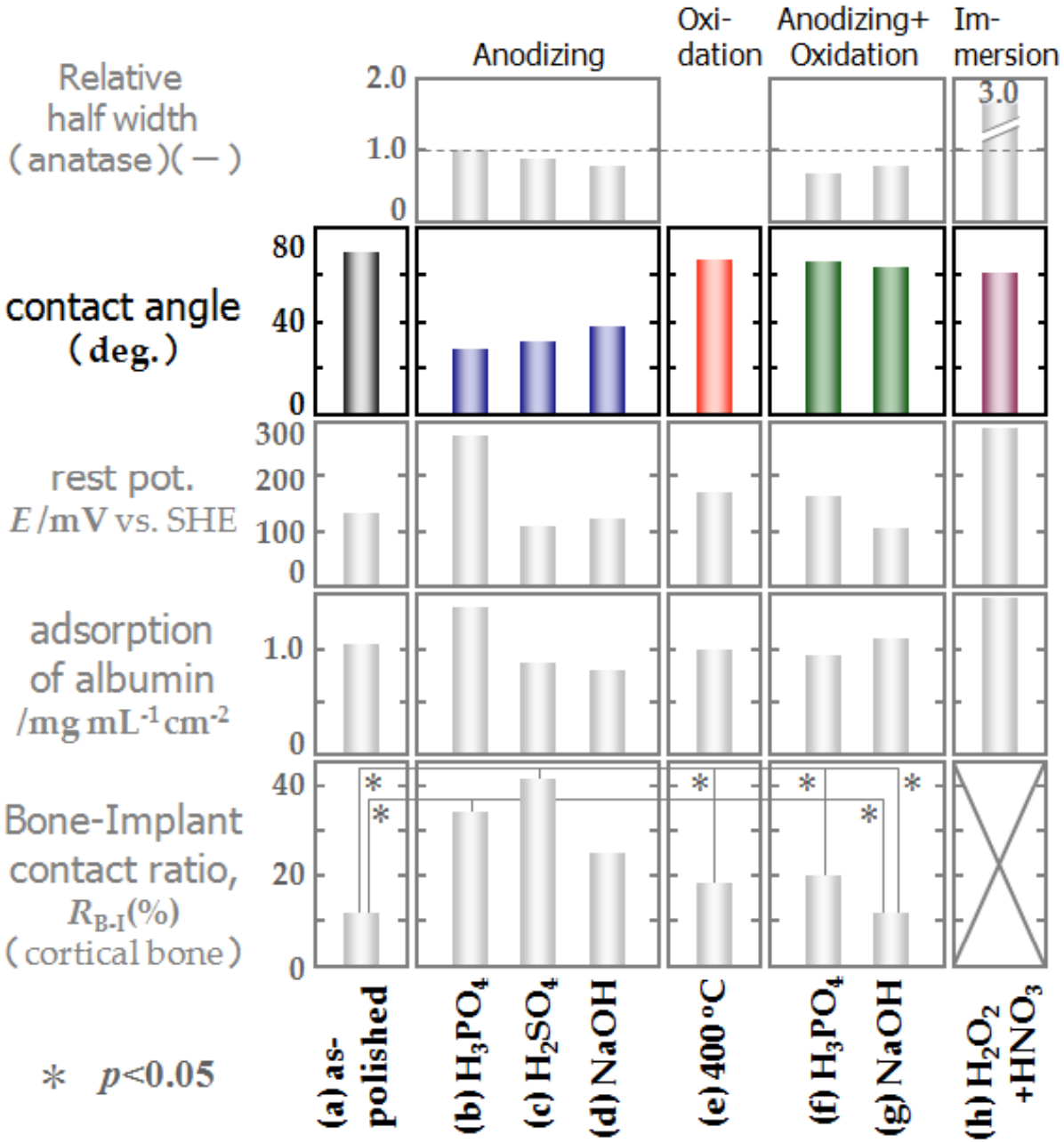
Anodizing+Oxidation

(f) 0.1M H_3PO_4, 70V+Vac., 400°C, 2h

(g) 0.1M NaOH, 50V+Vac., 400°C, 2h

(h) 8.8M H_2O_2+0.1M HNO_3 (80°C, 20min) — Immersion

Evaluation (hydrophilic, hydrophobic)



- Anodized TiO_2 films have small contact angle.
  （depend on the solution）
- Oxidation process increase the contact angle.
- TiO_2 gel films made by the immersion in the oxidizing solution have great angle.

(a) as-polished
(buffing, 0.05μm Al_2O_3)

(b) 0.1M H_3PO_4, 100V
(c) 0.1M H_2SO_4, 100V — Anodizing
(d) 0.1M NaOH, 80V

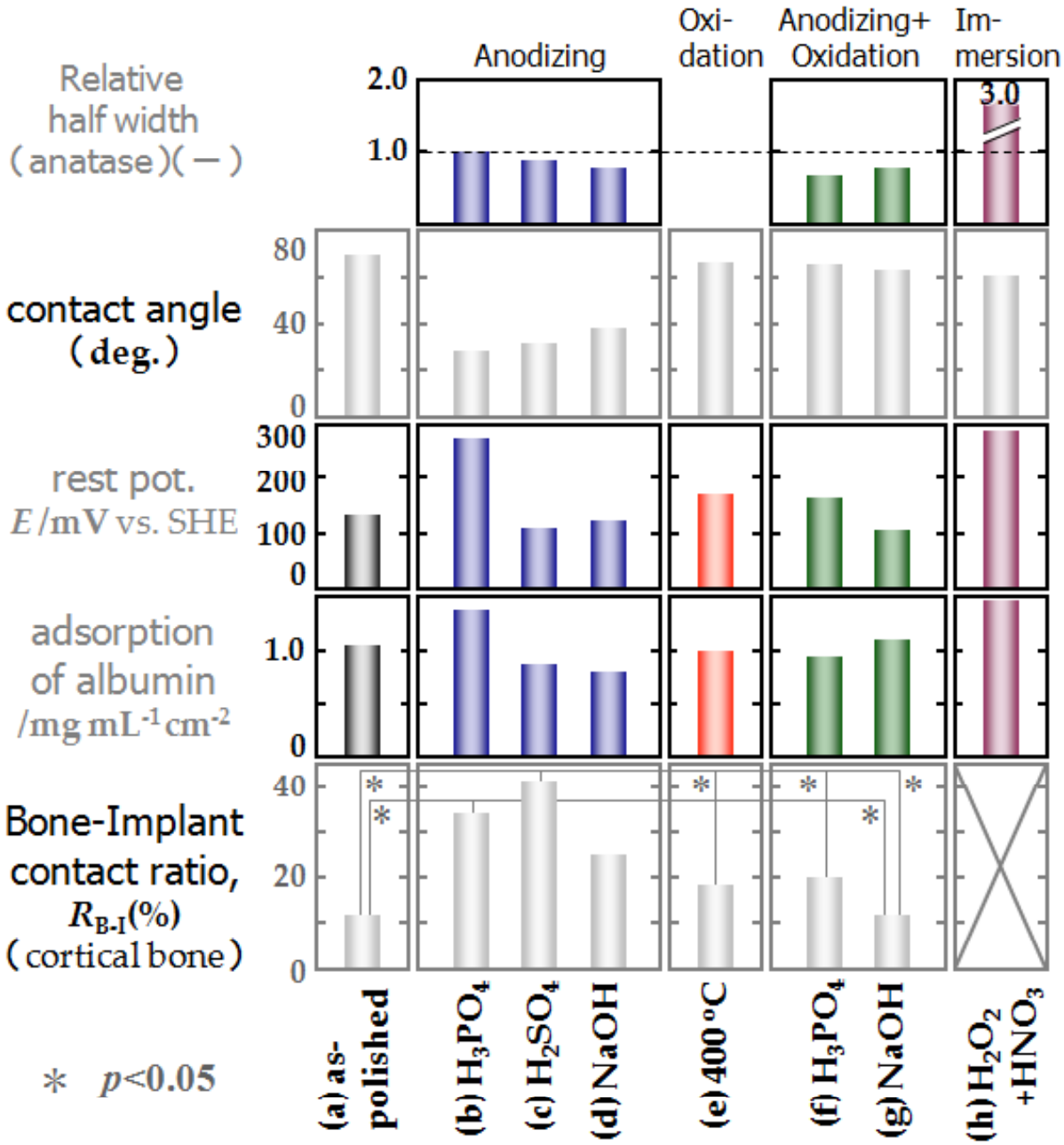
(e) Air, 400°C, 2h — Oxidation

Anodizing+Oxidation

(f) 0.1M H_3PO_4, 70V+Vac., 400°C, 2h
(g) 0.1M NaOH, 50V+Vac., 400°C, 2h

(h) 8.8M H_2O_2+0.1M HNO_3
(80°C, 20min) — Immersion

Evaluation (rest pot., albumin)



- Rest pot. of TiO_2 corresponds with the adsorption value of albumin.
- TiO_2 films anodized in H_3PO_4 and immersed in the oxidizing solution had high rest potential and adsorption value of albumin.

(a) as-polished (buffing, 0.05μm Al_2O_3)

(b) 0.1M H_3PO_4, 100V

(c) 0.1M H_2SO_4, 100V — Anodizing

(d) 0.1M NaOH, 80V

(e) Air, 400°C, 2h — Oxidation

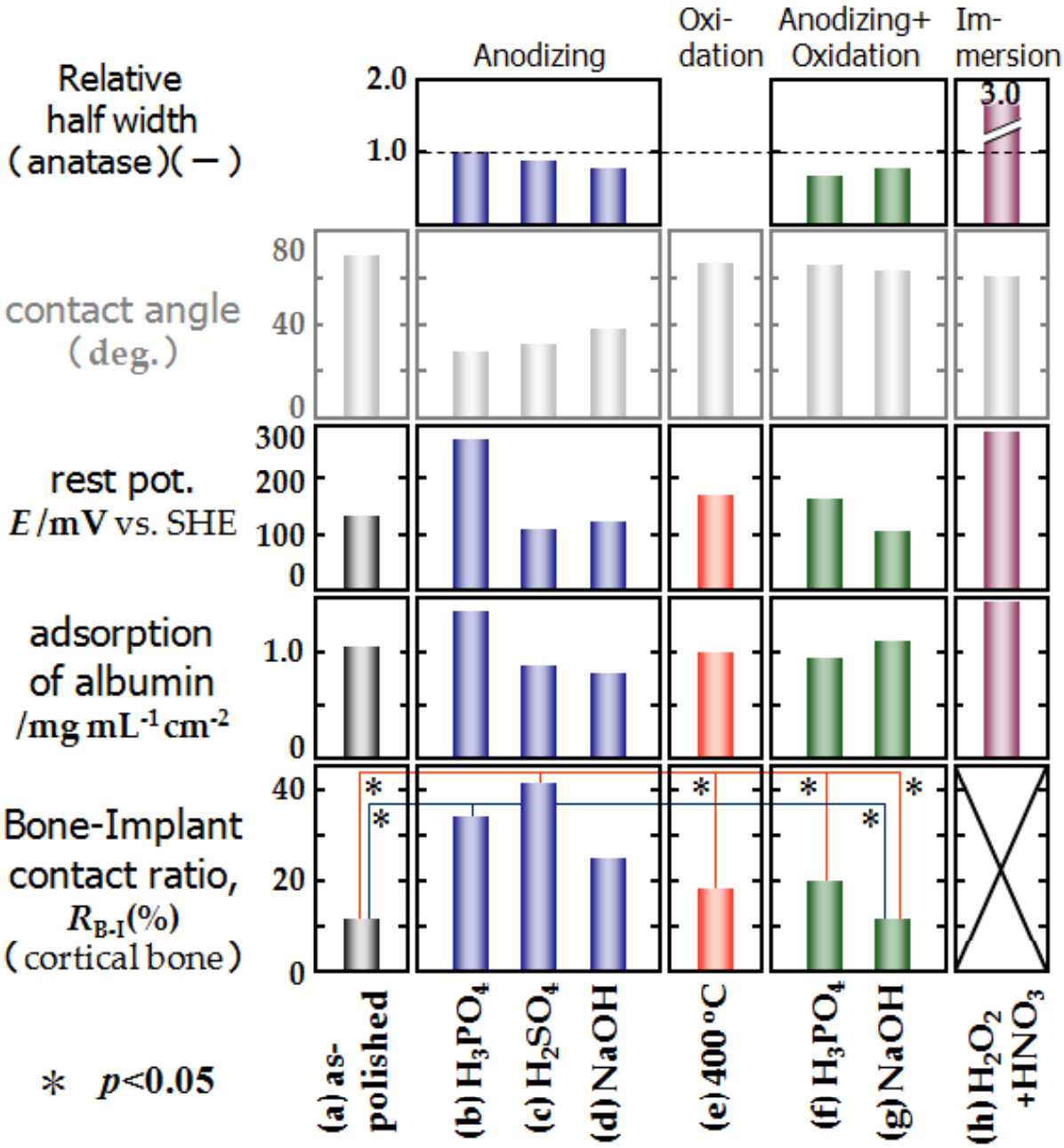
Anodizing+Oxidation

(f) 0.1M H_3PO_4, 70V+Vac., 400°C, 2h

(g) 0.1M NaOH, 50V+Vac., 400°C, 2h

(h) 8.8M H_2O_2+0.1M HNO_3 (80°C, 20min) — Immersion

Evaluation (osteoconductivity)



- The tendency of the rest potential, the crystallinity, and the albumin adsorption corresponds with the osteoconductivity.
- The osteoconductivity of TiO_2 films by immersed in the oxidizing solution appears promising.

(a) as-polished (buffing, 0.05μm Al_2O_3)

(b) 0.1M H_3PO_4, 100V

(c) 0.1M H_2SO_4, 100V — Anodizing

(d) 0.1M NaOH, 80V

(e) Air, 400°C, 2h — Oxidation

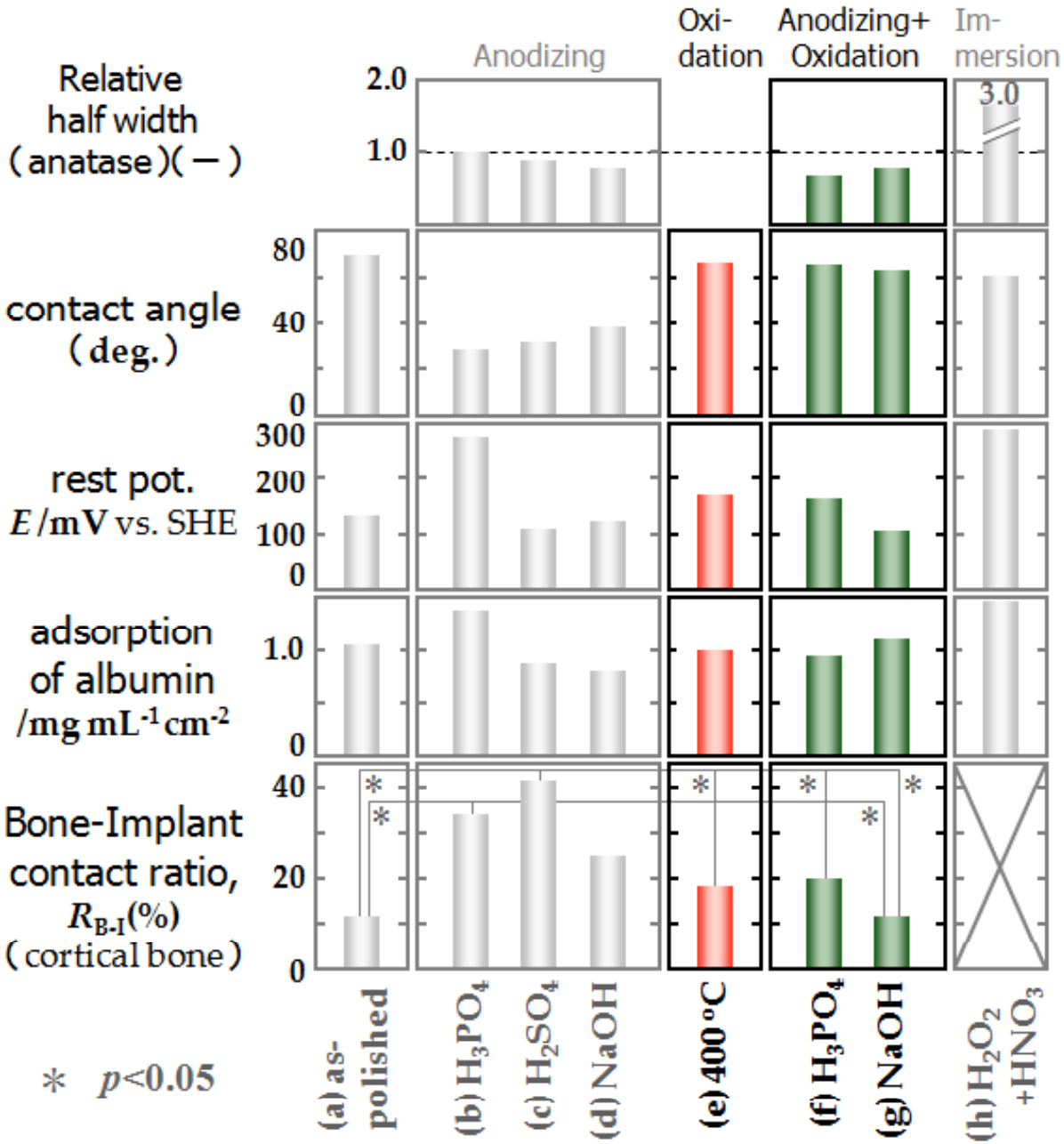
Anodizing+Oxidation

(f) 0.1M H_3PO_4, 70V+Vac., 400°C, 2h

(g) 0.1M NaOH, 50V+Vac., 400°C, 2h

(h) 8.8M H_2O_2+0.1M HNO_3 (80°C, 20min) — Immersion

Evaluation (anatase, rutile)



- In any evaluations, "Oxidation (Rutile)" = "Anodizing+Oxidation (anatase)"

(a) as-polished (buffing, 0.05μm Al_2O_3)

(b) 0.1M H_3PO_4, 100V

(c) 0.1M H_2SO_4, 100V — Anodizing

(d) 0.1M NaOH, 80V

(e) Air, 400°C, 2h — Oxidation

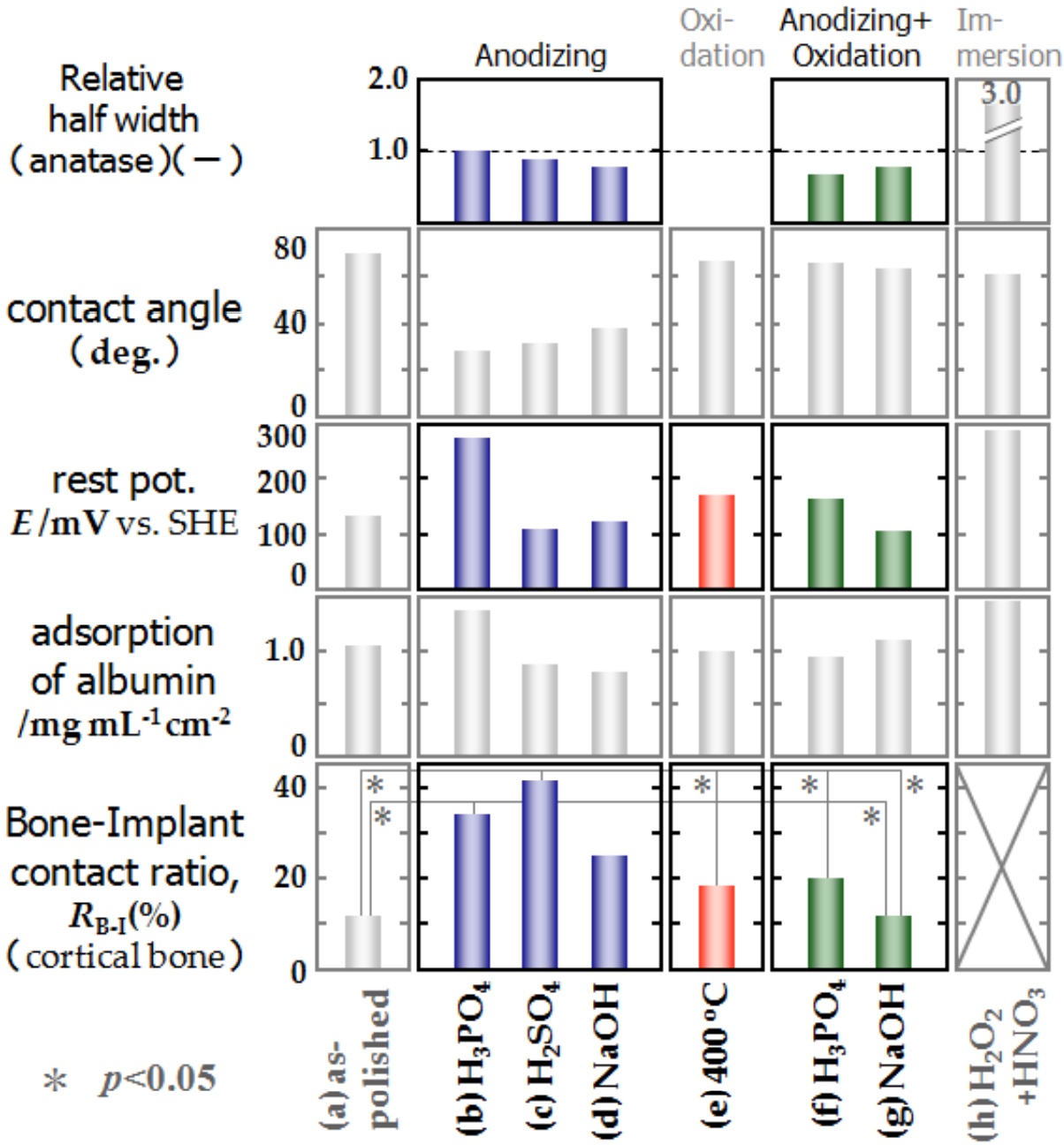
Anodizing+Oxidation

(f) 0.1M H_3PO_4, 70V+Vac., 400°C, 2h

(g) 0.1M NaOH, 50V+Vac., 400°C, 2h

(h) 8.8M H_2O_2+0.1M HNO_3 (80°C, 20min) — Immersion

Evaluation (anatase, rutile)



- The reason why $R_{B\text{-}I}$(anodizing) > $R_{B\text{-}I}$(oxidation) is NOT anatase has better osteoconductivity than rutile.
- The osteoconductivity depends on the coating process (hydro or pyro).

(a) as-polished (buffing, 0.05μm Al_2O_3)

(b) 0.1M H_3PO_4, 100V

(c) 0.1M H_2SO_4, 100V — Anodizing

(d) 0.1M NaOH, 80V

(e) Air, 400°C, 2h — Oxidation

Anodizing+Oxidation

(f) 0.1M H_3PO_4, 70V+Vac., 400°C, 2h

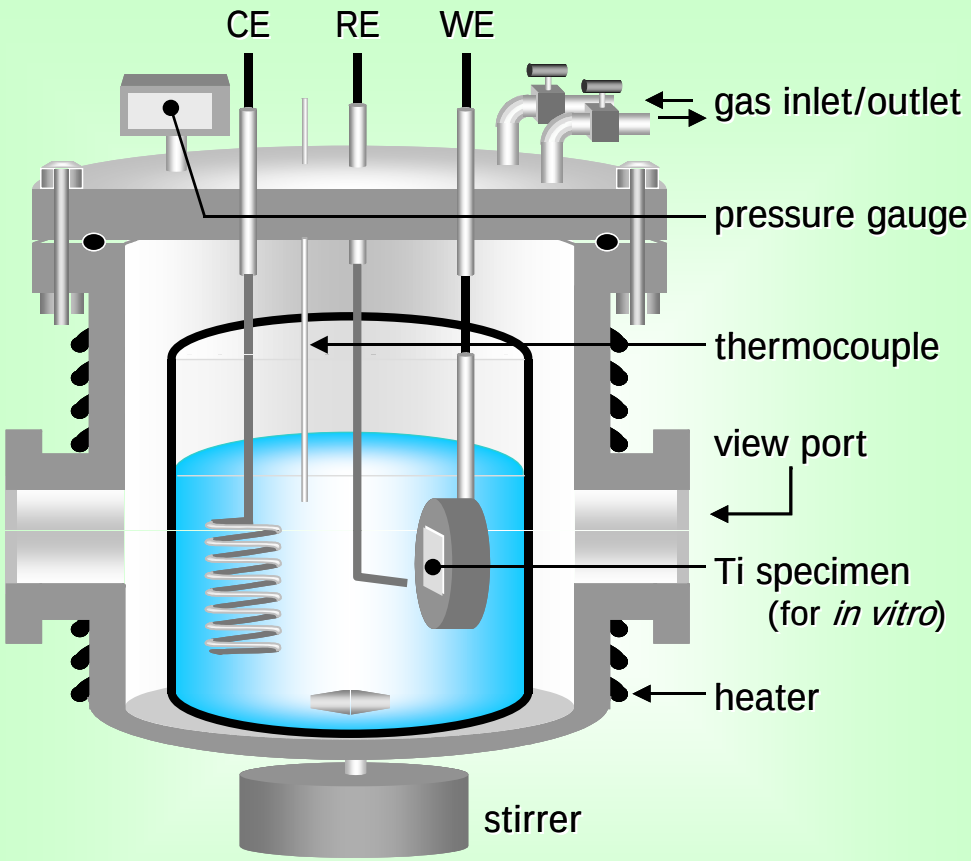
(g) 0.1M NaOH, 50V+Vac., 400°C, 2h

(h) 8.8M H_2O_2+0.1M HNO_3 (80°C, 20min) — Immersion

HAp/TiO_2 composite film

0.3mM $Ca(H_2PO_4)_2$ 0.7mM $CaCl_2$, pH 5.5

- Cathodic Electrolysis（HAp）
  - Potential：-1.3V ~ -9.3 V vs. Ag/AgCl
  - Time：900 s
- Anodic Electrolysis（TiO_2）
  - Potential：+8.7 V vs. Ag/AgCl
  - Time：900 s
- Single Square-Wave Electrolysis（TiO_2/HAp）
  - Anodic potential：+8.7 V vs. Ag/AgCl
  - Cathodic potential：-1.3V ~-9.3 V
  - Time：900 s + 900 s
- Pulse Electrolysis（TiO_2/HAp）
  - Anodic potential：+8.7 V vs. Ag/AgCl
  - Cathodic potential：-9.3 V
  - Cycle duty ratio
  - A30s-C30s, A30-C60, A60-C60
  - A300-C300（Total time：1800 s）



Bath Temp.：120℃, Intial Pres.：Ar 0.7 MPa

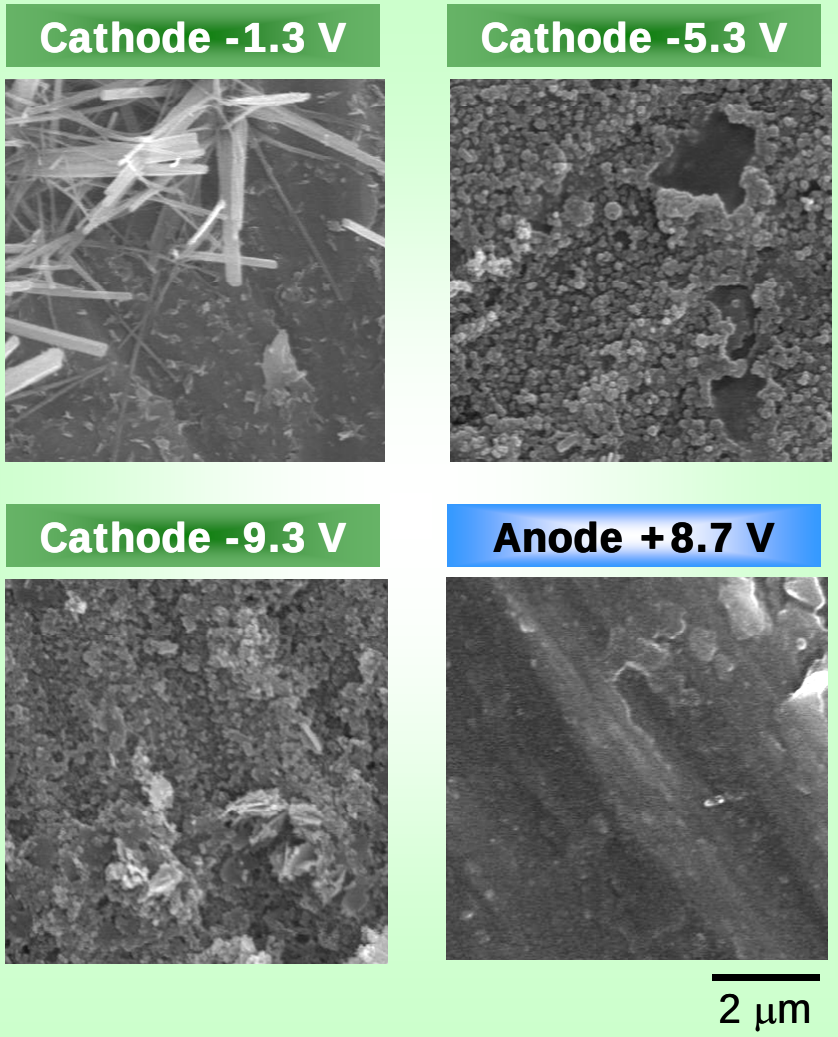
- Evaluation

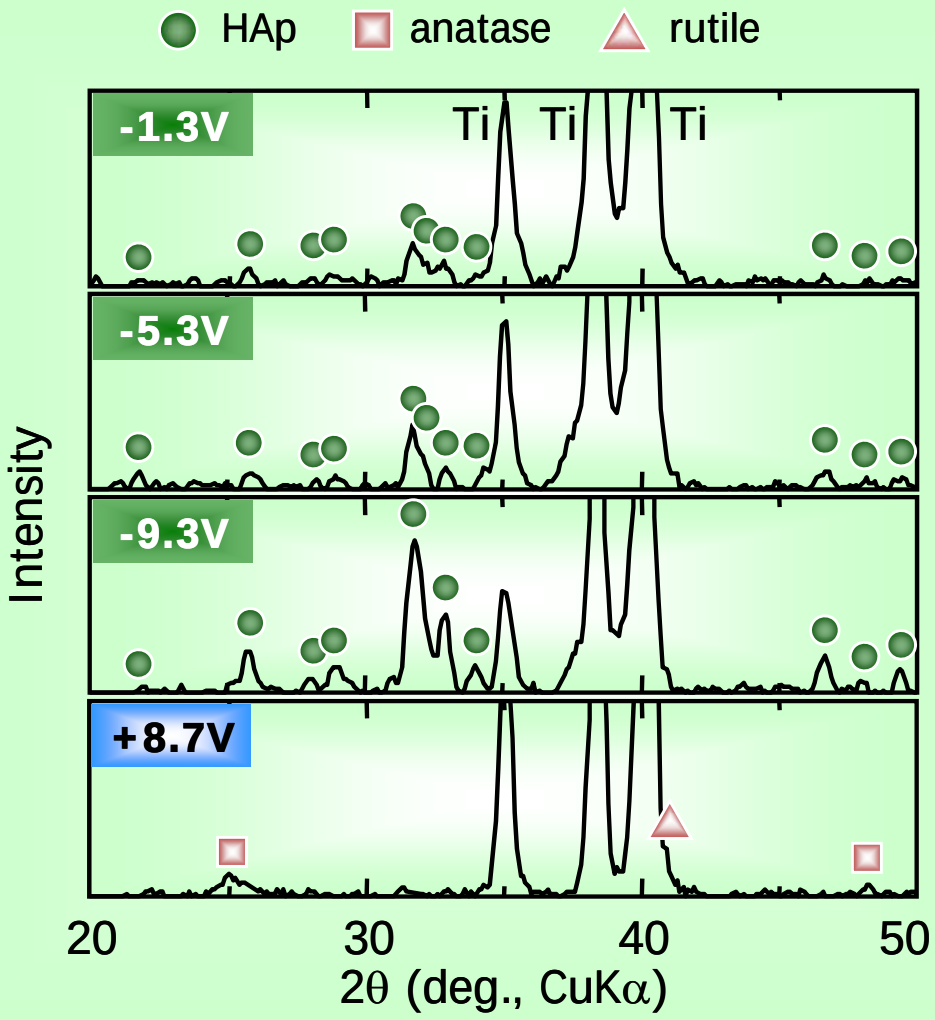
SEM-EDX, XRD → *in vivo*

Cathodic Electrolysis / Anodic Electrolysis

0.3mM $Ca(H_2PO_4)_2$ 0.7mM $CaCl_2$ pH 5.5, time : 900 s

Cathodic potential : **-1.3V ~ -9.3 V** vs. Ag/AgCl Anodic potential : **+8.7 V** vs. Ag/AgCl

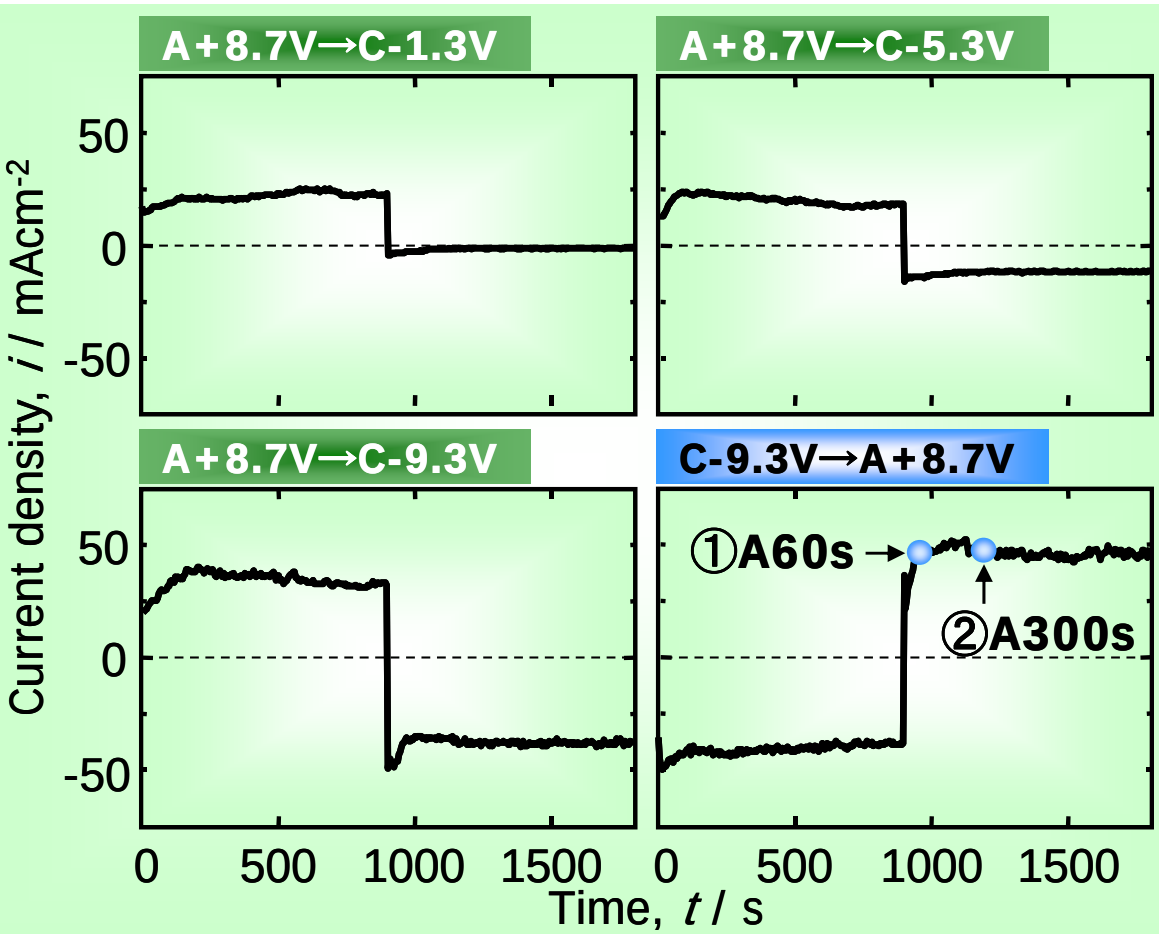


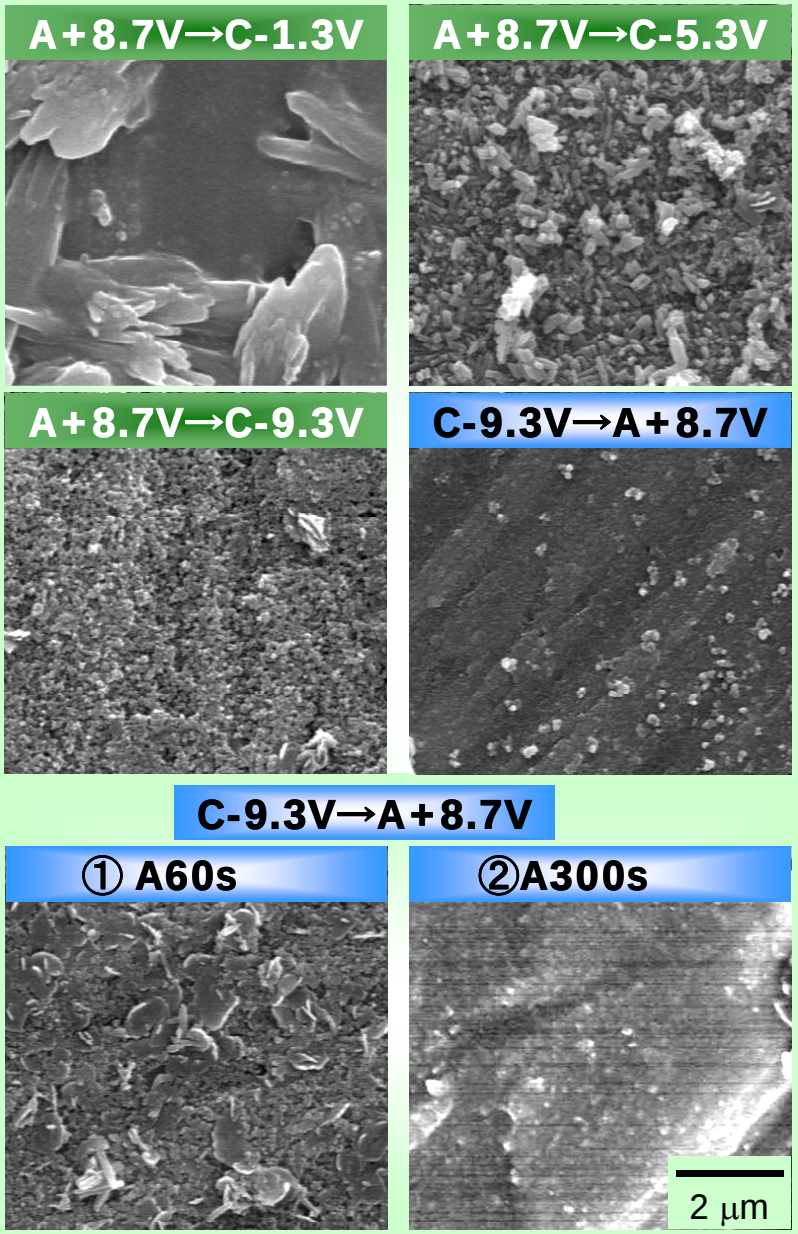


Single Square-Wave Electrolysis

0.3mM $Ca(H_2PO_4)_2$ 0.7mM $CaCl_2$ pH 5.5

Anodic potential: **+8.7V** vs. Ag/AgCl
Cathodic potential: **-1.3V, -5.3V, -9.3V**
Time: **900 s + 900 s**



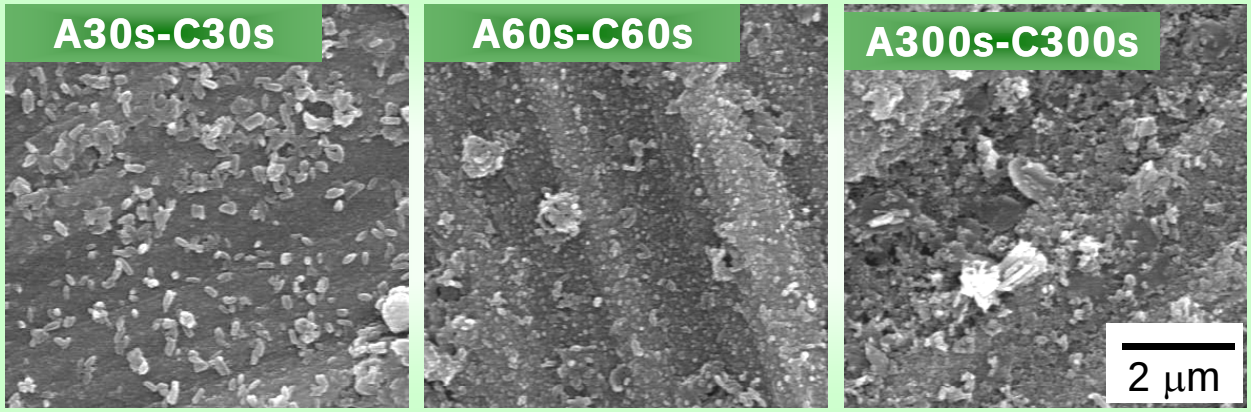


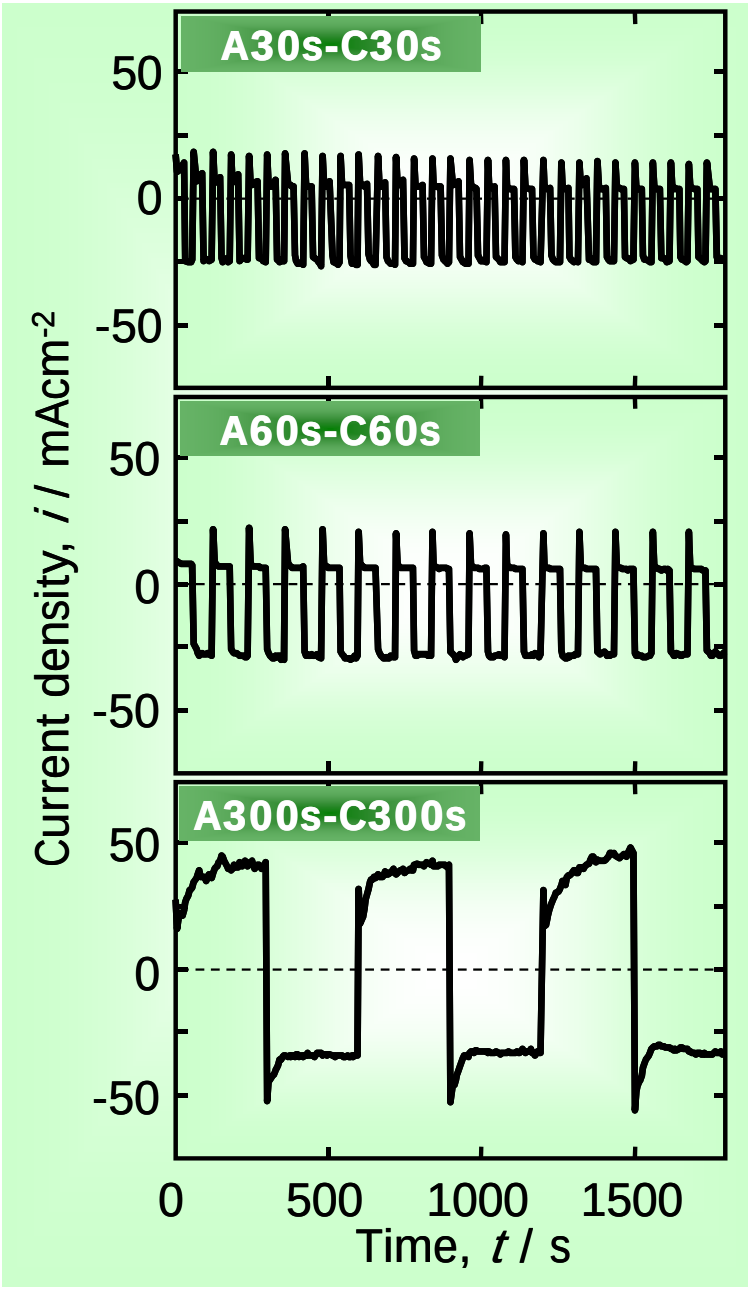
K. Kuroda, H. Shidu, R. Ichino, M. Okido: Mater. Trans., Vol. 48, No. 3, p. 322-327, (2007)

Pulse Electrolysis

0.3mM $Ca(H_2PO_4)_2$ 0.7mM $CaCl_2$ pH 5.5

Anodic potential: **+8.7 V** vs. Ag/AgCl
Cathodic potential: **-9.3 V**
Cycle duty ratio
A30s-C30s, A30-C60, A30-C90
(total electrolyzing time: 1800 s)





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K. Kuroda, H. Shidu, R. Ichino, M. Okido: Mater. Trans.,Vol. 48, No. 3, p. 328-331, (2007)
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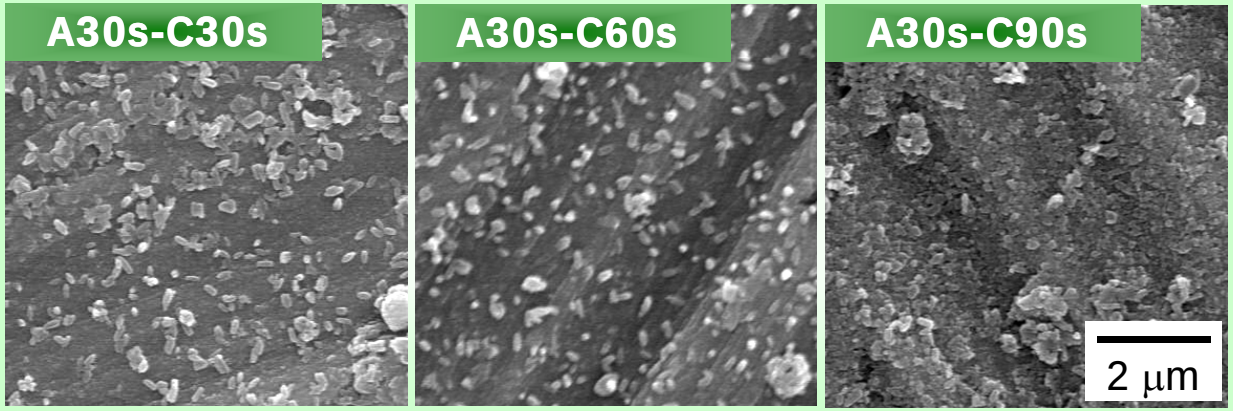
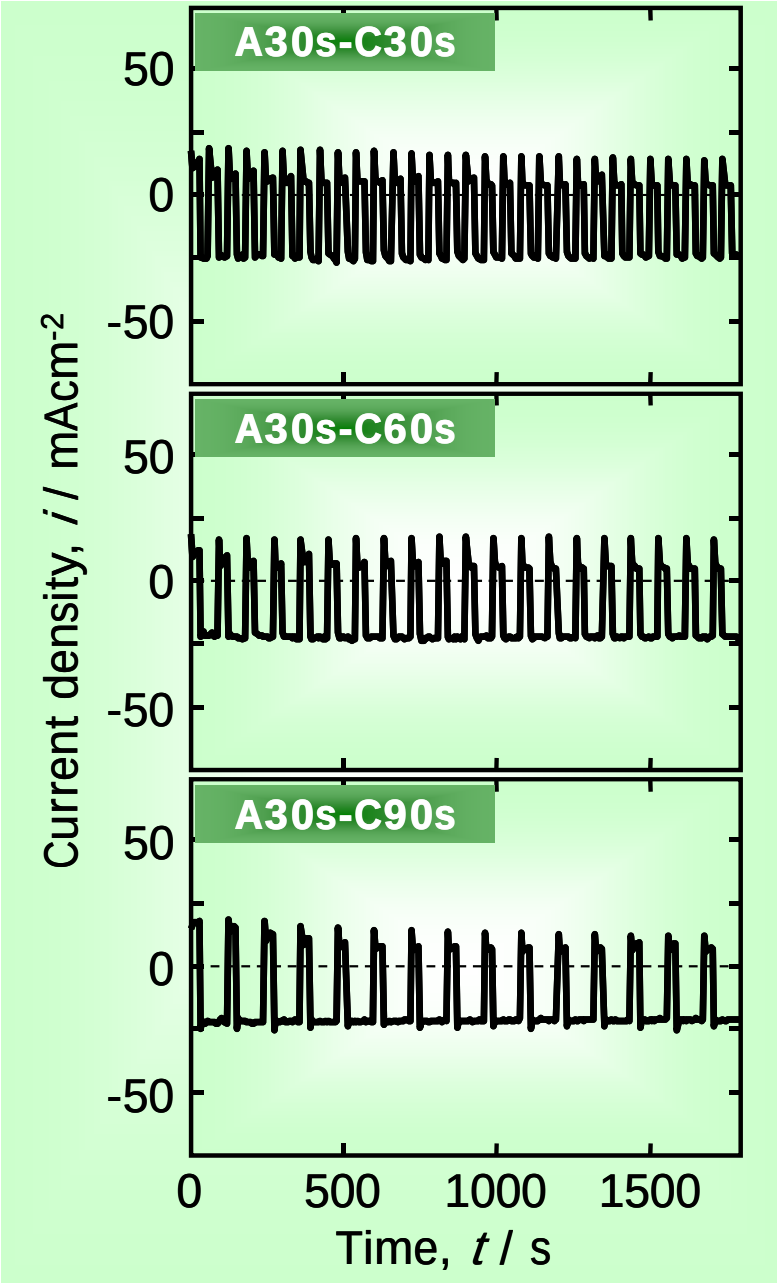
Pulse Electrolysis

0.3mM $Ca(H_2PO_4)_2$ 0.7mM $CaCl_2$ pH 5.5

Anodic potential: **+8.7 V** vs. Ag/AgCl
Cathodic potential: **-9.3 V**
Cycle duty ratio
A30s-C30s, A30-C60, A30-C90
(Total time: 1800 s)





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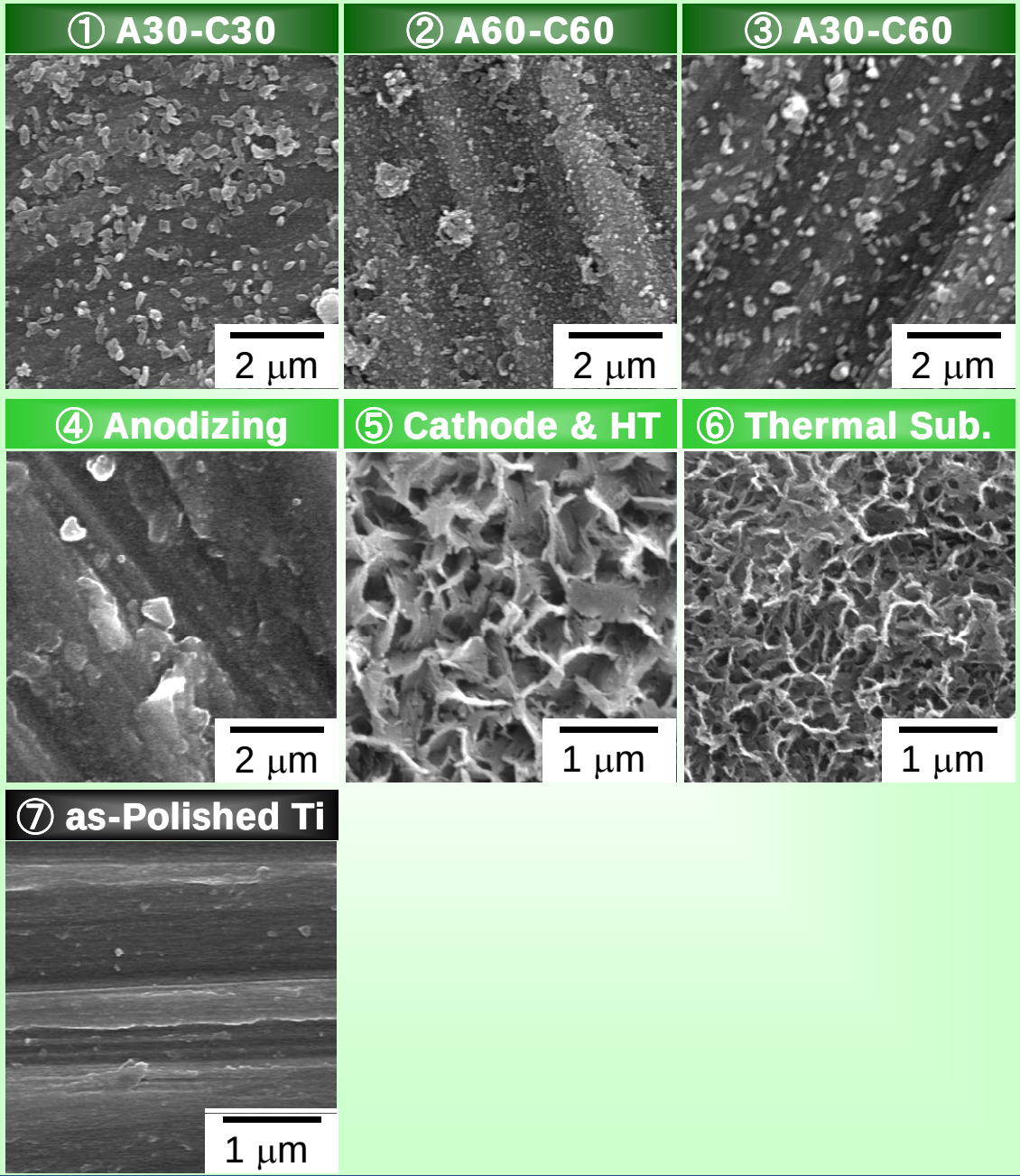
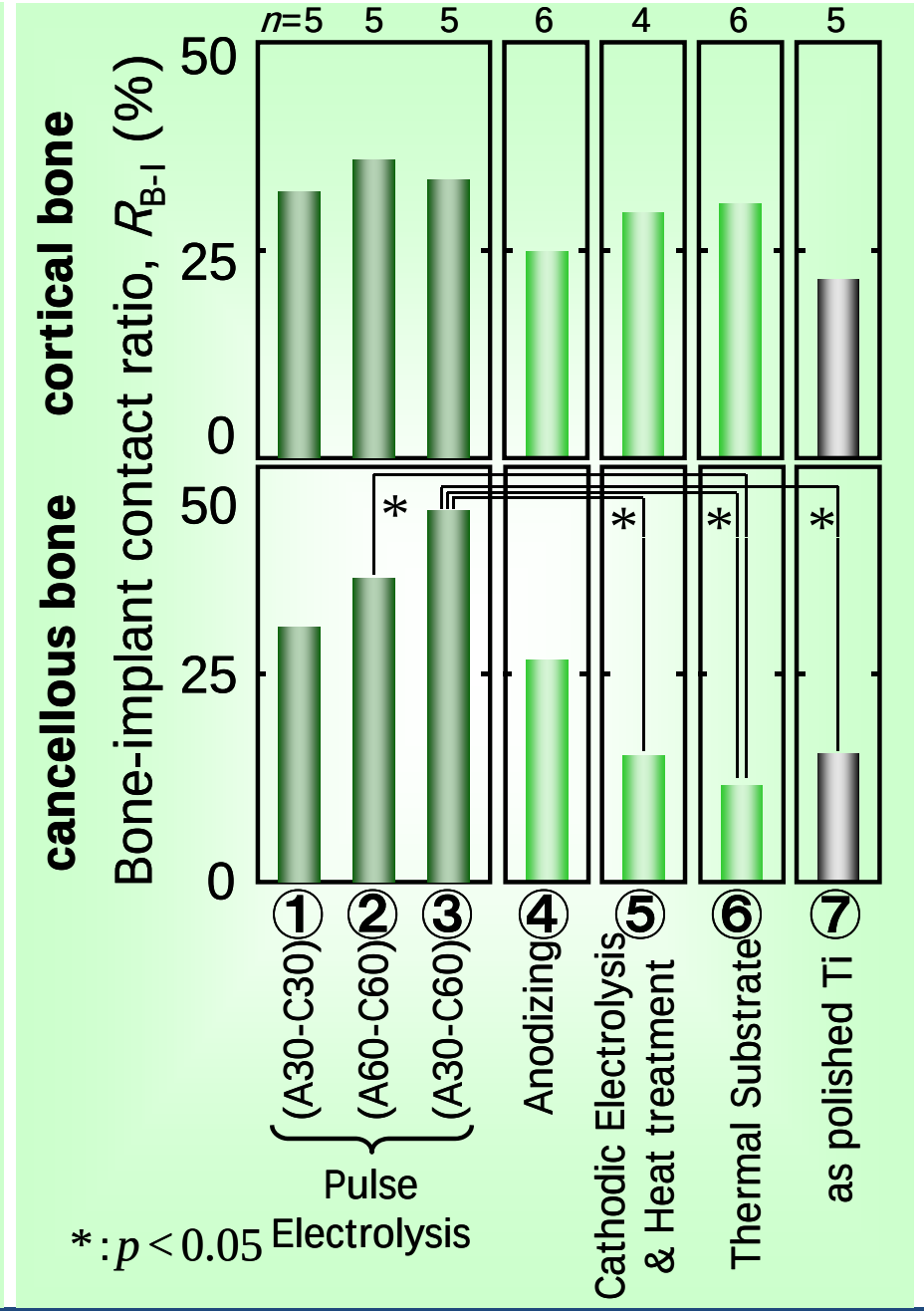
Evaluation


① A30-C30
② A60-C60
③ A30-C60
2 μm
2 μm
2 μm
④ Anodizing
⑤ Cathode & HT
⑥ Thermal Sub.
2 μm
1 μm
1 μm
⑦ as-Polished Ti
1 μm


n=5 5 5 6 4 6 5
cortical bone
cancellous bone
Bone-implant contact ratio, $R_{B\text{-}I}$ (%)
50
25
0
*
①(A30-C30)
②(A60-C60)
③(A30-C60)
Pulse Electrolysis
④ Anodizing
⑤ Cathodic Electrolysis & Heat treatment
⑥ Thermal Substrate
⑦ as polished Ti
*: $p < 0.05$

Concluding Remarks

- **Hydro-coating process,**
  thermal substrate method
  anodizing
  immersion in oxidizing acid solution
  of bioactive compounds, such as hydroxyapatite and titania was discussed. And also the titania coating using pyro-process, high temperature oxidation process, was explained.

- **The osteoconductivity of the coated bioactive compound films using hydro- and pyro-process was evaluated by in vitro and in vivo testing.**

- **The osteoconductivity of bioactive compound coatings by the hydro-process appears more promising than by the pyro-process.**