in vitro Study of HA and CMP Grit-Blasted and Acid Etched of Ti Surface

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Statement of Purpose: Grit-blasting method followed by acid etching has been developed to increase the surface roughness of the implant. Grit-blasting was done to generate macro-roughness, meanwhile acid etching to modify the micro-roughness of the specimens [1]. One of the examples of blasting materials is hydroxyapatite (HA) because HA is biocompatible and bioactive which can enhance the ossteointegration between implants and bone [2]. However, HA particles that are embedded on implant surfaces are removed using acid etching possibly due to the unfavorable effect of free HA particle. On the other hand, it is known that HA is able to accelerate proliferation and differentiation of cells [2]. In this study, bioactive HA and biodegradable calcium metaphosphate (CMP) grits were used as a blasting material. And *in vitro* comparative study of HA and CMP blasted and acid etched and non-etched samples were conducted. Cell viability, proliferation, and differentiation, and apatite-like formation in revised simulated body fluid (R-SBF) were examined.

Methods: Six sample groups were prepared in terms of blasting materials (HA, CMP) and etching reagents (non-etched, etched with HNO_3 and H_3PO_4), as shown in Table 1. Surface roughness was measured by laser surface profilometer. The amounts of residual particles (Ca and P contents wt%) on the surface were measured by EDX.

Table 1. 6 groups for in vitro study of HA and CMP blasted and etched samples

Group	Blasting Materials	Etching Reagent	Conc (vol%)	Etching Time	Residual Particles (wt%)		Ra (μm)
					Ca	P	
1	HA	-	-	1 min	16.9	8.59	6.746
2	HA	HNO_3	0.01	1 min	5.00	3.81	6.790
3	HA	H_3PO_4	0.01	1 min	10.08	5.72	6.753
4	CMP	-	-	1 min	3.70	7.47	6.251
5	CMP	HNO_3	1	1 min	0.88	1.79	6.178
6	CMP	H_3PO_4	1	1 min	1.19	2.5	6.231

* where, Ra values of etched and non-etched samples were not significantly different (about 6.7 μm for HA-blasted and 6.2. μm for CMP-blasted samples)

R-SBF test was done by immersion for 1, 7, and 21 days. SEM and Thin Film XRD were used to examine the surface of samples. Cell viability and proliferation test were performed by MTT Assay for 1 and 3 days, and the optical densities were measured at 570 nm in an ELISA reader. Alkaline phosphatase (ALP) staining for 7 days and RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR) were done to measure cell differentiation behavior.

Results / Discussion: After 1 week in R-SBF, apatite-like layer was found on HA-blasted specimens (group 1-3), meanwhile not found on CMP-blasted specimens (group 4-6). This result showed that HA on Ti surface promotes the apatite formation. From TF-XRD results, the intensity of apatite peaks in group 1 and 3 was somewhat higher compared to group 2, which showed that the more amount of residual HA on the surface leads to the faster apatite formation. After 3 weeks in R-SBF, all specimens have apatite layer on their surface, except group 4. In CMP-blasted and etched samples (group 5-6), although it was observed the presence of apatite, the amount of apatite formation was still low and not fully covered the surface. From MTT-Assay test, HA-blasted samples showed better cell viability and proliferation compared to CMP-blasted samples after 1 and 3 days (Fig.1.). There was no significant difference observed by comparing the etched and non-etched samples of each HA and CMP-blasted ones. But in CMP-blasted and etched with H_3PO_4, significant increase in cytotoxicity was observed after 3 days. This may be caused by the change in surface chemistry properties due to H_3PO_4 treatment. From ALP Staining images, no significant difference in osteoblastic differentiation between 6 group samples was observed. From RT-PCR results, group 1 showed better osteoblastic differentiation compared to other groups (2-6). This result suggested that non-etched HA-blasted samples were better in terms of cell differentiation compared to etched ones, and CMP-blasted samples, either etched or non-etched.

Conclusions: HA- and CMP-blasted residues on Ti surfaces affected the apatite-like formation in R-SBF. Non-etched HA-blasted samples showed the faster apatite formation compared to etched ones. HA-blasted samples (etched and non-etched) showed better cell viability and proliferation than CMP-blasted samples. And the cell differentiation of non-etched HA-blasted samples was better compared to others. That is, the biological performance of Ti samples is slightly different and depends upon the blasting materials, acid etching, and etching agents.

References:

[1] H.J. Ronøld, J.E. Ellingsen, Biomaterials 23 (2002) p4211-4219
[2] L.L. Hench and June Wilson, *An Introduction to Bioceramics* (1993) p140-180.

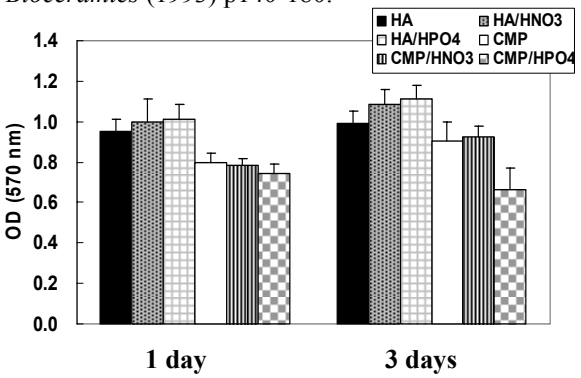

Fig.1.Cell proliferation after 1 and 3 days