The effects of ultraviolet photofunctionalization of implant on bone graft in critical one-wall defects around implant: a pilot study in Beagle dogs

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(Directed by Prof. Hong Seok Moon, D.D.S.,M.S.D.,Ph.D.)

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This certifies that the dissertation thesis of

Min Young Kim is approved.

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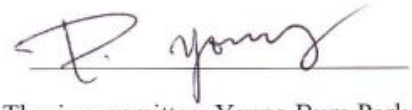

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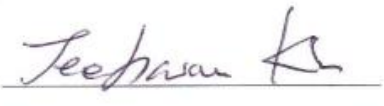

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June 2014

감사의 글

모든 것이 합력하여 선을 이룬다는 것을 깨닫게 해주시며 항상 좋은 길로 인도해 주시는 하나님께 가장 먼저 감사를 드리며 영광을 돌립니다.

이 논문의 시작에서 완성까지 전 과정에 걸쳐 세심하고 끊임없는 지도와 격려, 조언을 해주신 문홍석 교수님께 진심으로 감사를 드립니다. 또한, 논문의 작성과 심사에 귀중한 조언과 격려를 해주신 박영범 교수님, 이재훈 교수님, 김지환 교수님, 정한성 교수님, 김성태 교수님께도 깊은 감사를 드리며, 바쁘신 가운데에서도 항상 따뜻한 관심과 조언으로 지켜봐 주신 정문규 교수님, 한동후 교수님, 이근우 교수님, 심준성 교수님께도 감사 드립니다. 그리고 힘든 시편제작과 계측에 많은 도움을 준 박상현 연구원, 이채은 연구원과 실험을 도와주었던 최현민 선생과 보철과 후배 김홍준에게도 감사의 마음을 전합니다. 또한, 지면을 통해 일일이 언급하지는 못하지만 저에게 도움과 격려를 주신 모든 분들께도 다시 한번 감사 드립니다.

마지막으로, 무엇보다도 언제나 한결같은 사랑과 내조로 든든한 버팀목이 되어주고 나를 믿고 따라준 가장 사랑하는 나의 아내 혜원과 무조건적인 사랑과 끊임없는 기도로 격려해주시는 아버지, 어머니, 장인어른, 장모님과 누나, 매형, 처남에게도 진심으로 감사의 마음을 전하면서 이 기쁨을 함께하고 싶습니다.

2014 년 6 월

김민영 드림

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Abstract

The effects of ultraviolet photofunctionalization of implant on bone graft in critical one-wall defects around implant: a pilot study in Beagle dogs

Min Young Kim D.D.S., M.S.D.

(Directed by Prof. Hong Seok Moon, D.D.S.,M.S.D.,Ph.D.)

Purpose: The conversion of implant surface from bioinert to bioactive by UV photofunctionalization can result better osseointegration and osteogenesis capabilities of titanium surface. The purpose of this study was to compare and evaluate, through histomorphometric and radiological assessment, the effects of UV photofunctionalization on an implant placed over a critical defect area with and without a bone graft.

Materials &Methods: Four female beagle dogs (body weight 10 kg, age 12 months)

were first divided into bone graft and control groups. Each group was again subdivided into UV-treated and control groups. The mandibular premolars in each dog were extracted. 12 weeks after extraction, implants were placed according to the condition of each group. Four and 12 weeks after implantation on left and right mandible, the dogs were sacrificed. The specimens were prepared for histomorphometrical and micro-computed tomographic analyses.

Results: In both the 4-week and 12-week groups, UV-treated implant surfaces showed better osseointegration than on SA implant surfaces, while both implant surfaces were in direct contact with bone. The UV photofunctionalization barely showed any effect on implant surfaces placed over the critical defect without bone graft. After 12 weeks, however, UV photofunctionalization increased the amount of new bone formation. In implant surfaces placed over the critical defect with bone graft, UV photofunctionalization increased BIC and new bone formation at the initial stage (4 wks).

Conclusion: Based on the results of this study, UV photofunctionalization on the surface of implants placed over large critical defects with bone graft aid initial osseointegration and osteogenesis. If the limitations of this study are addressed in subsequent experiments, it will be possible to examine UV photofunctionalization's long-term positive effect on implants placed over large critical defects with bone grafts.

Key words: UV photofunctionalization, Bone graft, Critical defect, Implant, BIC

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I. Introduction

In modern implant dentistry, implant therapy is known as the most effective treatment of choice for edentulous areas and is regarded as the first considered treatment for a missing tooth. The success of dental implants depends on how directly new bone

formation occurs on titanium surface after implant placement.[1] The initial amounts of osseointegration and osteogenesis are crucial factors in the success rate of implant therapy.[2,3] In order to increase initial osseointegration and osteogenesis, therefore, various methods have been researched and developed. In 1997, Wang et al. reported that hydrophilicity was much higher when ultra-violet (UV) photofunctionalization was applied as a titanium surface treatment.[4] In recent studies, Ogawa et al. researched the effects of UV photofunctionalization on titanium surface and found that UV photofunctionalization increased hydrophilicity, eliminated hydrocarbon, and also improved the electrostatic status of the surface via electropositive charging. They thus concluded that conversion of implant surface from bioinert to bioactive, yielded better osseointegration and osteogenesis on titanium surface.[5-7]

The success of implant therapy also depends on bone condition of the site during implant placement, as surgery is not always under ideal conditions. When there is a bone defect around the site for implant placement, there is insufficient bone support from the surrounding bones and partial surfaces are not fully covered with bones, leading to a failure of the surgery in the long term. The degree of both osseointegration and osteogenesis is high enough even without bone graft when the distance between implant and bone is about 1 mm (Boticelli et al. 2003),[8] when there is a horizontal gap within 2 mm between implant and bone, normal gap healing is achieved without any bone graft (Jung et al. 2007).[9] In the case of a large bone defect area, a bone graft is carried out along with an implant placement and the bone graft becomes a scaffold around the implant. Among several types of bone graft materials, some support migration of

osteoblast and osteoinductive materials to the implant surface, thereby increasing the probability of success in the long term.[10-12] Per Snyder's case in 2012, the success of implant surgeries with radiographically perfect osseointegration was reported without long-term proof of any clinical inflammation and infection when implants of 4 mm horizontal gaps were placed with bone grafts without membrane.[13] However, some reported that bone graft materials arrested new bone formation at the outset by blocking proliferation of osteoblast.[14-16] Nevertheless, once grafting materials are absorbed and eventually disappear, the space gets filled with new bone so that the level of osseointegration and osteogenesis finally increases.[17,18]

The success rate of an implant placement with a bone graft can be increased by overcoming negative impacts of bone grafting materials on osseointegration and osteogenesis at the beginning of the placement stage. Since there already exists a clinical study on effects of UV photofunctionalization on implant surfaces at insufficient bone condition,[6] this experiment was designed to examine whether positive clinical results also showed optimistic outcomes histomorphometrically and radiologically through animal testing with beagle dogs. The purpose of this study was to compare and evaluate effects of UV photofunctionalization on an implant placed over a critical defect area with a bone graft through histomorphometric and radiological assessments. The models in the present study were built with critical defects of 5 mm, larger than ones in a previous study evaluating positive effects of UV photofunctionalization on implant surface and osseointegration in experiments using rats[19] and in a clinical study examining benefits of implants with UV photofunctionalization.[6] Regardless of using bone graft material, the

study also examined effects of UV photofunctionalization on implant surface in terms of optimistic effects for each case of bone defect that did not heal over the duration of the study. The experiment was conducted with the hypothesis that osseointegration and osteogenesis on an implant surface will be enhanced or influenced when UV photofunctionalized implants are placed for two groups using DFDBA after artificially creating a critical one-wall defect that will not heal over a certain period of time.

II. Materials and Methods

2.1. Experimental animals

In this study, four female beagle dogs of twelve months old (weighing approximately 10 kg) were used. As a pre-study preparation, scaling and plaque control were performed for periodontal health. After treatment, they were fed with liquid foods to prevent masticatory trauma during healing. Animal selection, management, surgical protocol, and all experiments were reviewed and approved by the Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea (no. 2010-0362).

2.2. Experimental implants

A total of 24 SA (sandblasting with alumina and acid etching) surface-treated internal type implants (Osstem implant system, TS II SA Fixture, Busan, Korea) 3.5 mm in diameter and 8.5 mm in length were used in this study (Fig. 1). All implants used in the experiment were made simultaneously and stored in a sealed container, being kept minimally exposed to the air immediately before placement.

A. B. * Figure provided by manufacturer

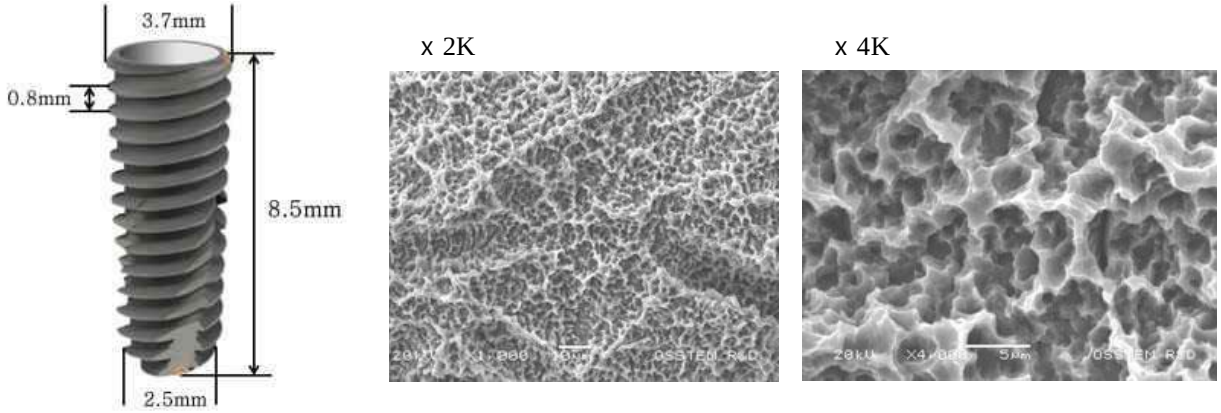


Fig. 1 A. Design of implant fixture (TS III SA Fixture, Osstem)
B. Scanning electron microscopy images of SA surface

2.3. Ultraviolet photofunctionalization

Photofunctionalization was performed by treating implants with UV light for 15 minutes using a photo device (TheraBeam Affiny, Ushio Inc., Tokyo, Japan) immediately before implantation (Fig.2).

A. B. C.

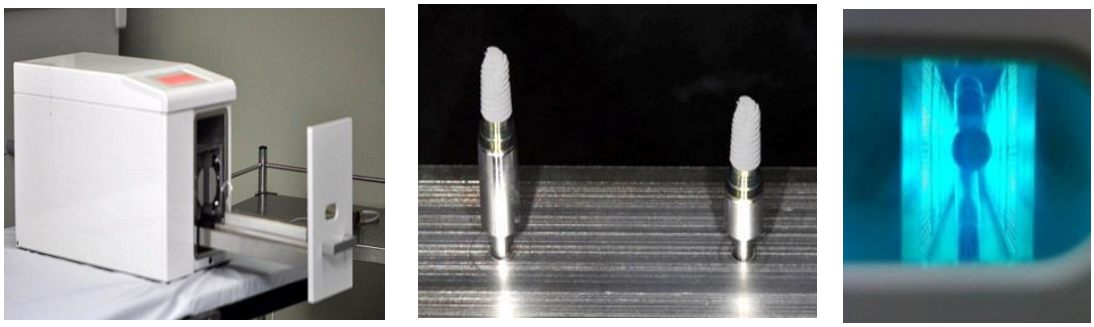


Fig. 2 Photofunctionalization of implant fixture surface by ultraviolet light

A. TheraBeam Affiny device (TheraBeam Affiny, Ushio, Tokyo, Japan)

B. Implant fixtures mounted on tray

C. Process of UV treatment on implant fixture during 15 minutes

2.4. Graft materials

Demineralized freeze dried bone allograft (SureOss-D ,Demineralized Cortical Bone Powder, Hans Biomed Corp., Seoul, Korea) 200~850㎛ in particle size was used for grafting at bone defect (Fig.3).

Fig. 3 SureOss™ (Demineralized Cortical Bone Powder, Hans Biomed Corp., Seoul, Korea)

2.5. Experimental groups design

First, all implant placement sites were divided into bone graft groups and control groups, each group then subdivided again into UV treatment group and control group. The UV untreated group with bone graft was set to group BG, the UV treated group with bone graft was set to group UV/BG, the UV treated group without bone graft was set to group UV, and finally the UV untreated group without bone graft was set to group Control. Eight SA surface implants were used for group UV/BG, eight SA surface implants for group BG, four SA surface implants for group UV, and lastly four SA surface implants for group Control. In this study, the sample sizes of group UV and group Control were reduced since the UV photofunctionalization effect has already been proved in previous studies. Implants were placed symmetrically to reduce differences in the sites by matching the initial states. For two beagle dogs, four implants (two per dog) were placed from posterior area of the 1st premolar in the right and left side of mandible. For another beagle dog, two implants were placed from posterior area of the 1st premolar in both sides of mandible and from posterior area of the 3rd premolar in both sides of mandible for the rest of the beagle dogs (Tables. 1 & 2). The distance between each implant was 10 mm and the experiment was carried out using a split-mouth design.[20] The implants placed in the left side of mandible had a healing period of four weeks (4 week group) and the other implants placed in the right side of mandible had a healing period of twelve weeks (12-week group) (Fig. 4).

Table.1 Experimental groups classified by UV light treatment for groups with bone graft.

	Left (4 weeks healing period)				**Right (12 weeks healing period)**			
Placement location	PM1	PM2	PM3	PM4	PM1	PM2	PM3	PM4
Group	BG	UV/BG	BG	UV/BG	BG	UV/BG	BG	UV/BG
	UV/BG	BG	BG	UV/BG	UV/BG	BG	BG	UV/BG

* BG: UV-untreated implant with bone graft, UV/BG: UV-treated implant with bone graft

Table.2 Experimental groups classified by UV light treatment for groups without bone graft

	Left (4-week healing period)				**Right (12-week healing period)**			
Placement location	PM1	PM2	PM3	PM4	PM1	PM2	PM3	PM4
Group	Control	UV			Control	UV		
			Control	UV			Control	UV

* Control: UV-untreated implant without bone graft, UV: UV-treated implant without bone graft

Fig.4 Diagram of experimental design protocol.

2.6. Surgical procedure

All of the surgical treatments were carried out under general anesthesia. Both mandibular premolars (from first to fourth premolar) were extracted atraumatically. Twelve weeks after the extraction of four teeth (P1, P2, P3, P4) on right side of mandible, sequential drilling for implant placement was performed. The distance between centers of implants was 10 mm. A one-wall bony defect in cuboid shape was then formed for each group. Those defects were uniformly 3 mm in depth and 5 mm in width (bucco-lingual and mesio-distal). The center of implant was then placed on the edge of the cuboid (Fig. 5). Photofunctionalization was performed for 15 minutes using a photo device immediately before implantation. In the case of grafting, 0.25 cc DFDBA was gently packed into each bone defect until it filled the entire cavity (Fig. 6). One week after the surgical procedure, stitching out was done. The same processes were executed on left side of mandible eight weeks later. The beagle dogs were sacrificed after four weeks.

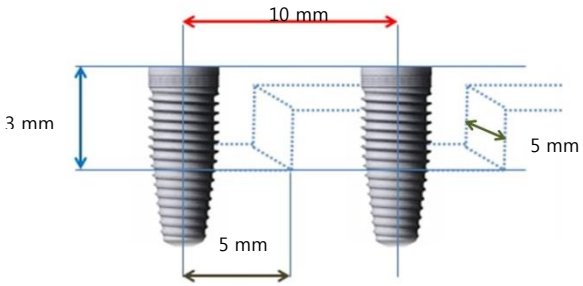
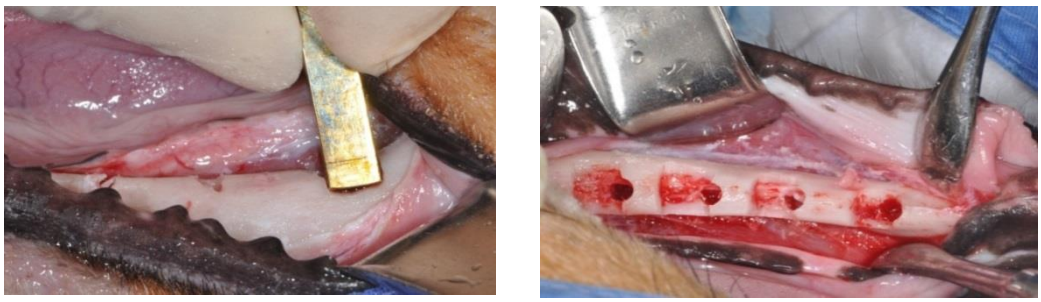


Fig.5 Schematic diagram of experimental design

A. B.



C. D.

Fig.6 Clinical views of surgical procedure

A: Forming the standardized defects, B: Drilling for implant placement and forming the defects

C: Implants placed with bone graft, D: Wound closure with suture

2.7. Fabrication of histologic specimens

After buffering of the tissue samples with neutral formalin fixation for two weeks, micro-CT was taken. Next, the samples were dehydrated in increasing grades of ethanol and subsequently infiltrated in Technovit 7200 resin (Heraeus Kulzer, Dormagen, Germany). Following the embedding in the acrylic resin, the blocks were polymerized and sectioned in the mesio-distal plane using a cutting-grinding unit (Exakt Exakt 300, Heraeus Kulzer, Norderstedt, Germany). The 400 μm thick units thus obtained were further reduced using EXAKT grinding machine (KULZER EXAKT 400CS, Germany) to a final thickness of about 30 μm. The tissue samples were processed for ground sectioning according to methods described by Donath & Breuner and the sections were stained in H&E (Hematoxylin & Eosin) for light microscopic examination.[21]

2.8. Histomorphometrical analysis

The stained histologic specimens were scanned and captured using optical microscopy at 12.5x and 50x magnification and then histomorphometrically measured using Image-Pro Plus 4.5 (Media Cydernetrics, Silver Spring, Maryland, USA). At first the actual zone of formed defect was set as a region of interest (ROI) by sectionalizing the region on histologic specimens. Then, the ratio for bone-to-implant contact (BIC, %), new bone area formed in defect area (new bone area, %), remaining graft material area in defect area (graft material area, %) and resorption area in defect area (resorption area, %)

were calculated in ROI (Fig. 7). BIC was measured at three consecutive threads in ROI as well as in the corresponding ROI located at the same threads in the opposite side, used as an inner control site. The measurements in the inner control site were used to revise those in ROI (Fig. 8). New bone area ratio and remaining graft material area ratio were calculated as a percentage of the area occupied by each one in ROI (Fig. 9). Lastly, resorption area ratios were obtained as a percentage of the area in which bone filling did not occurr in ROI (Fig.10).

A. B.

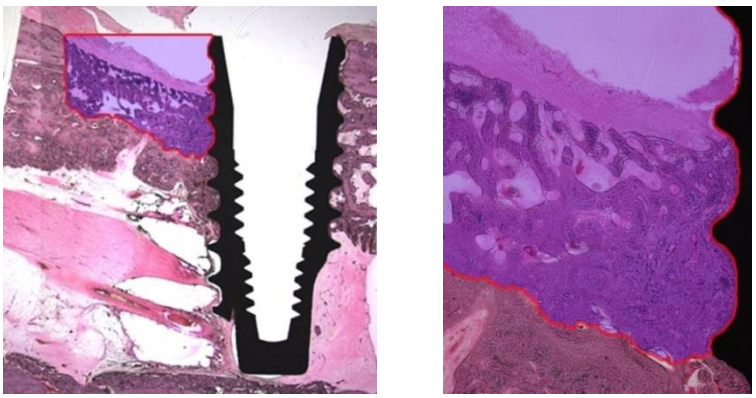


Fig.7 Histologic images showing experimental and opposite side, Red box → ROI

A: H&E stained image showing experimental and opposite side as inner control site (X12.5)

B: Region of interest (ROI) in experimental site, (H&E stained, X50.0)

A. B.

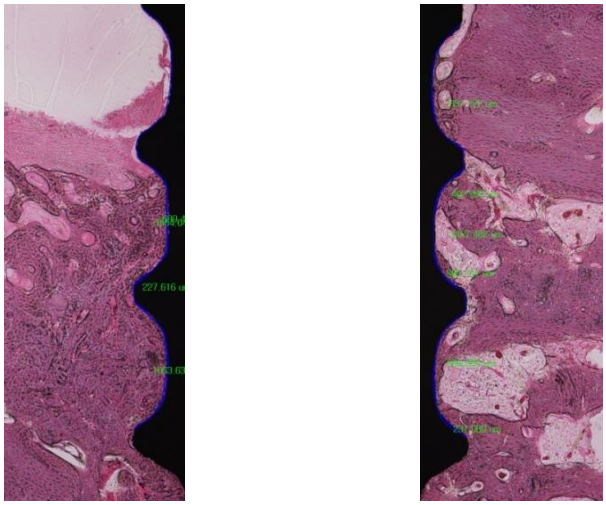


Fig.8 Measurement of bone-to-implant contact (%)

A: Measurement of BIC in experimental site

B: Measurement of BIC in opposite side as inner control site

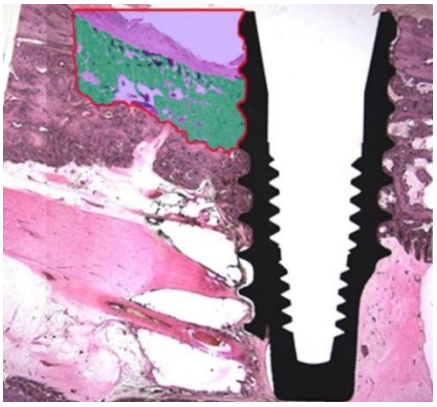


Fig.9 Measurement of new bone area (%) or remaining graft material area (%) → Green zone

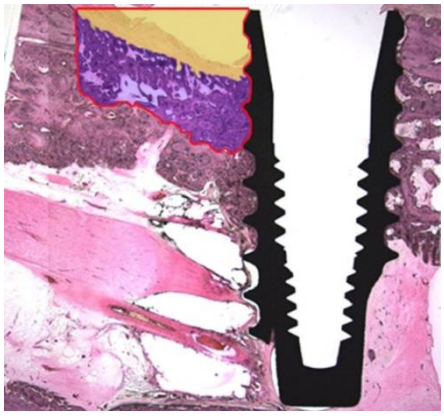


Fig.10 Measurement of resorption area (%) → Yellow zone

2.9. Micro-computed tomographic analysis

A micro computed tomography scanner (Skyscan 1076, SkyScan, Aartselaar, Belgium) was used to measure the percentage of the amount of new bone formation around implants. Scans were made at a medium resolution of 18 μm with Al 0.5 mm filter at 100 Kv and 100 μAv and the amount of bone was compared using the difference of blackening of images (with black level of images). The new ROI was set up in a rectangular parallelepiped 3.5 mm in width (mesio-distal) from the center of implant, 3.0 mm in length (bucco-lingual), and 3.0 mm in height. The amount of bone was measured in this area. As area of the same size was also set up at the inner control site from which measurements were used to revise those in ROI (Fig. 11).

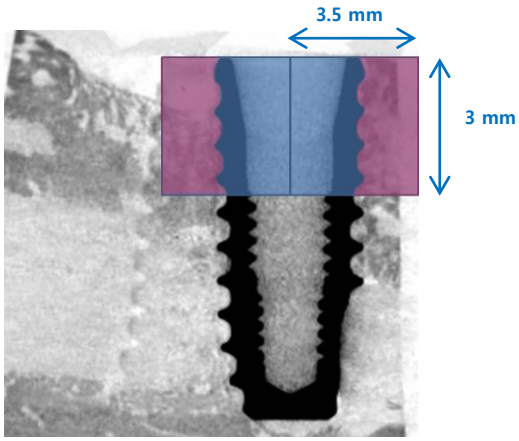


Fig.11 Bone volume measuring range in micro CT image

2.10. Statistical analysis

The BIC in the opposite side, used as the inner control site, was measured to verify the difference in osseointegration due to UV treatment. The independent samples t-test was used with the UV-treated and UV-untreated groups. Next, the ratios for BIC, new bone area, remaining graft material area, resorption area, and micro-CT value were compared to verify the difference between groups UV and Control. The same measurements were carried out for groups BG and UV/BG. The Wilcoxon rank sum test was used as a non-parametric statistical method to evaluate the data due to the small sample size and large standard deviations. All calculations were performed using a specific statistical program (SPSS Ver. 18.0, IBM Co., Somers, NY, USA), and the level of significance was set at 5**%.**

III. Results

3.1. Histomorphometrical findings

3.1.1. Comparison of bone to implant contact ratio based on UV treatment

BIC was measured at three consecutive threads in the inner control site ROI in the opposite side. BIC was calculated as a percentage of the length in direct contact with the implant surface in ROI of the opposite side. Measurements were divided into two groups according to whether UV treatment had been applied. The independent samples t-test was used on two groups (Table. 3 & Fig. 12). Based on comparison of mean values, the BIC in the UV-treated group was higher than in the UV-untreated group at both fourth and twelfth weeks. There was no statistically significant difference between the two groups using independent samples t-test.

Table.3 The mean of bone-to-implant contact at 4 weeks 12 weeks in the inner control site

Group		**Inner control site BIC**	
		4 wks	**12 wks**
UV X	Mean	61.73	48.68
	SD	23.26	22.34
	SE	8.79	7.06
UV O	Mean	68.33	56.94
	SD	19.54	40.87
	SE	8.74	20.43
	[§]p-value	0.083	0.664

[§] The p-values were calculated using an independent sample t-test.

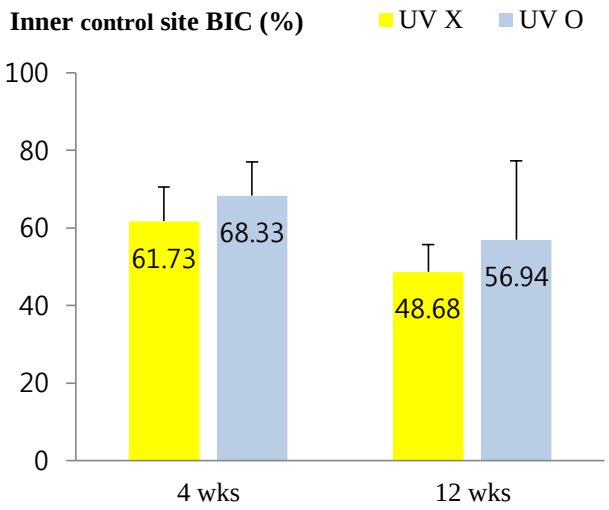


Fig.12 The mean of bone-to-implant contact at 4 and 12 weeks in the inner control site

3.1.2. Comparison of bone-to-implant contact ratios in control and experimental groups

3.1.2.1 Comparison of cases without bone graft

In the control and UV groups, BIC was measured at three consecutive threads in ROI, which had been set up for histomorphometrical analysis, and at those corresponding threads in the opposite side as an inner control site (Fig. 13). BIC was calculated as a percentage of the length in direct contact with the implant surface in ROI. The inner control site measurements were used to revise those in ROI. The relative BIC ratio values for each group were obtained by dividing the ROI measurements by the inner control site ROI measurements. The mean value and standard deviation of relative values were then calculated. The Wilcoxon rank sum test was performed on these data, yielding values of

0.50 in group Control at 4 weeks, 0.57 in group UV at 4 weeks, 1.13 in group Control at 12 weeks and 1.07 in group UV at 12 weeks. There was no significant difference between the two groups (Table. 4 & Fig. 14).

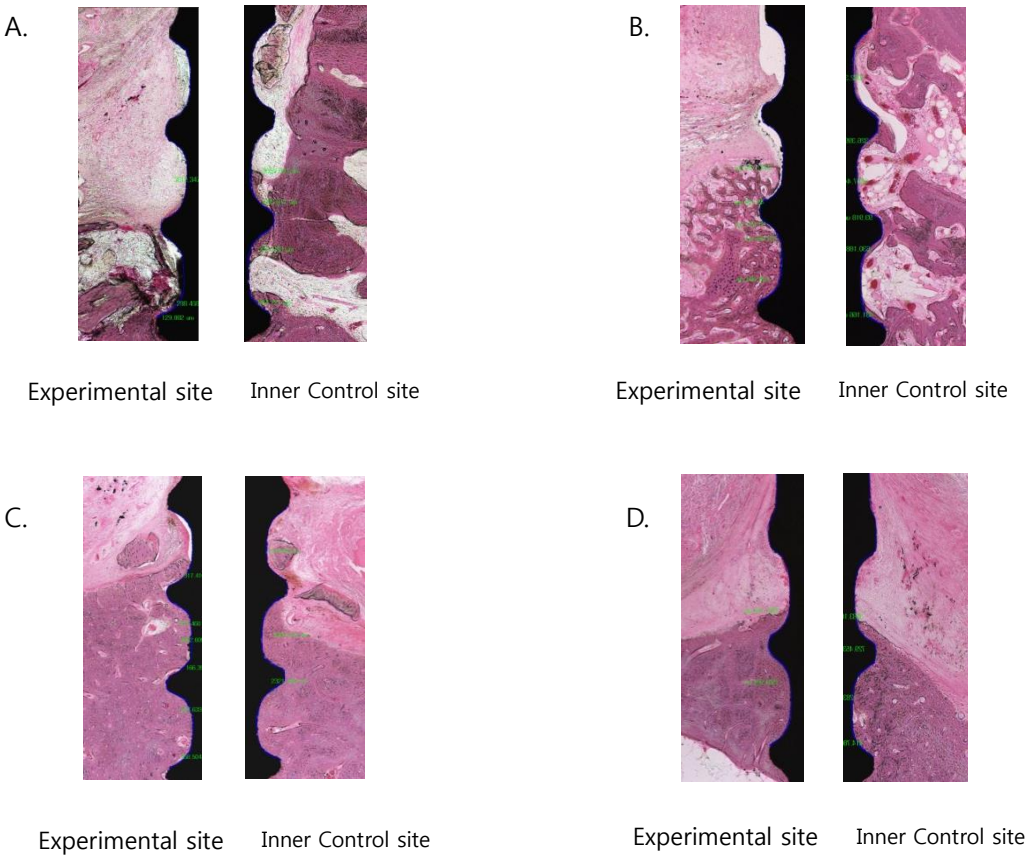


Fig.13 The representative of each group at 4 and 12 weeks.
(H&E stain, Original magnification : X50)
A: Control 4, B: UV 4, C: Control 12, D: UV 12

Table.4 The mean of relative bone-to-implant contact ratios at 4 and 12 weeks.

Group		Relative BIC ratio	
		4 wks	12 wks
Control	Mean	0.50	1.13
	SD	0.45	0.58
	SE	0.19	0.24
UV	Mean	0.57	1.07
	SD	0.33	0.03
	SE	0.23	0.02
[§]p-value		0.737	0.505

[§] The p-values were calculated using the Wilcoxon rank sum test.

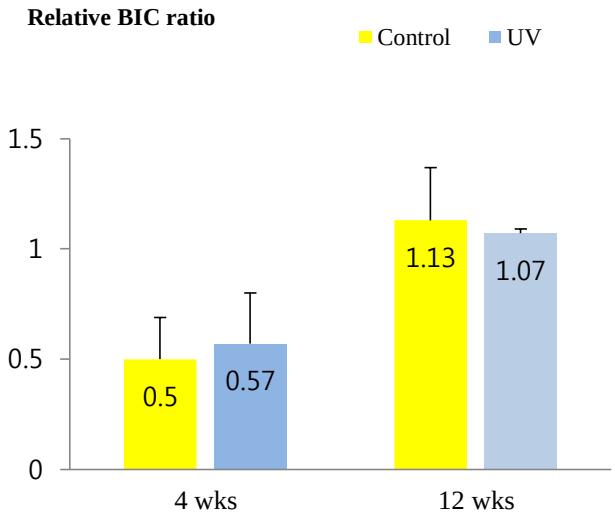


Fig.14 The mean of relative bone-to-implant contact ratios at 4 and 12 weeks

3.1.2.2 Comparison of cases with bone graft

In groups BG and UV/BG, BIC was measured using the same methods described earlier (Fig. 15). The relative BIC ratio values of each group were obtained and the mean value and standard deviation of relative values then calculated. The Wilcoxon rank sum test was performed using these data, yielding values of 0.08 in group BG at 4 weeks, 0.32

in group UV/BG at 4 weeks, 0.36 in group BG at 12 weeks, and 0.12 in group UV at 12 weeks. There was no significant difference between the two groups (Table. 5 & Fig. 16).

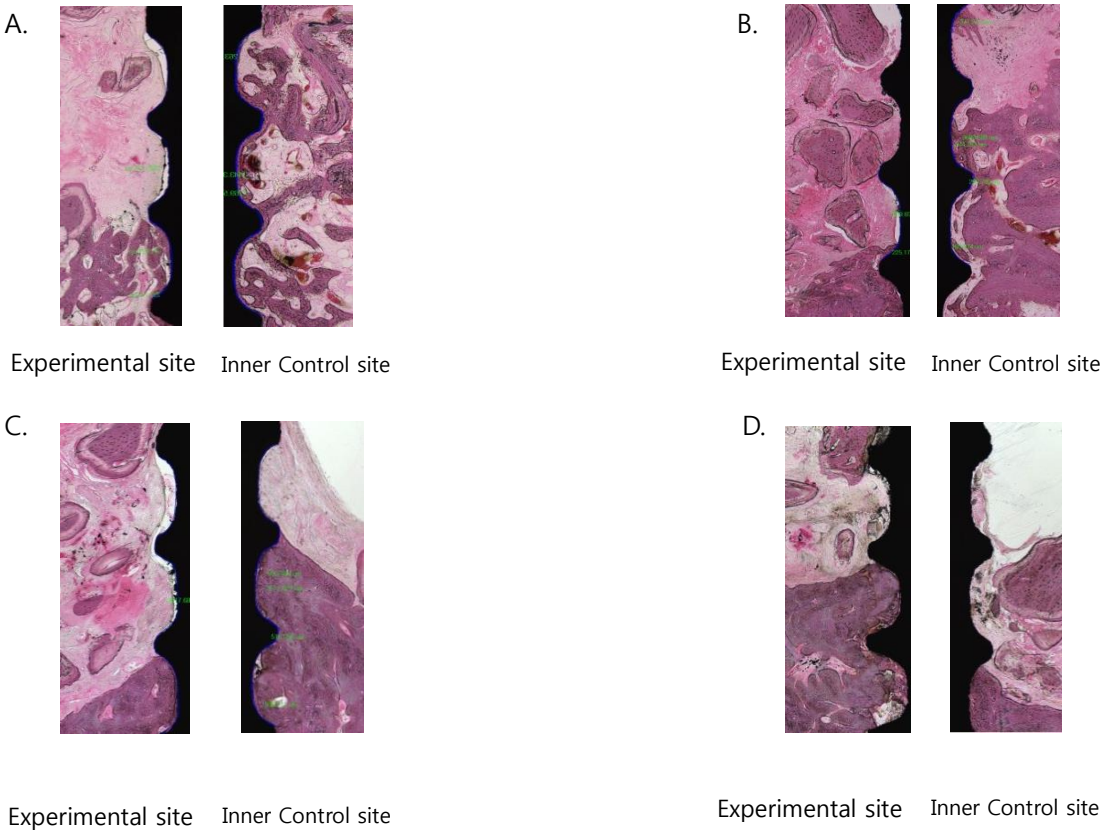


Fig.15 The representative of each group at 4 and 12 weeks.
(H&E stain, Original magnification : X50)
A: BG 4, B: UV/BG 4, C: BG 12, D: UV/BG 12

Table.5 The mean of relative bone-to-implant contact ratios at 4 and 12 weeks.

Group		Relative BIC ratio	
		4 wks	12 wks
BG	Mean	0.08	0.36
	SD	0.15	0.31
	SE	0.08	0.16
UV/BG	Mean	0.32	0.12
	SD	0.29	0.24
	SE	0.15	0.12
	[§]**p-value**	0.271	0.814

[§] The p-values were calculated using the Wilcoxon rank sum test.

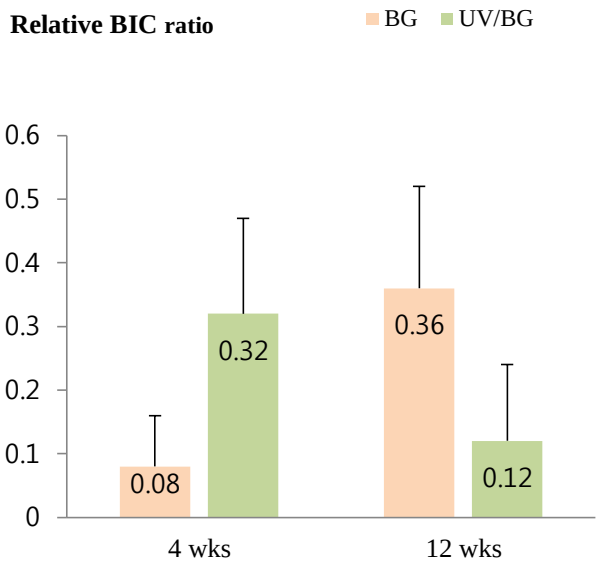


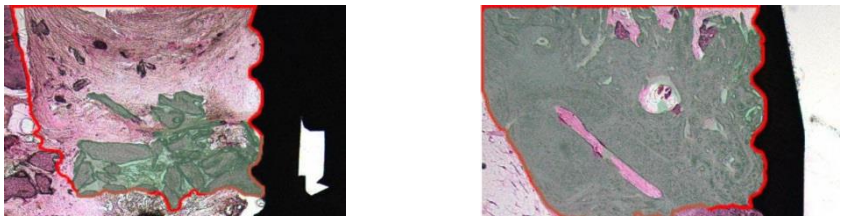
Fig.16 The mean of relative bone-to-implant contact ratios at 4 and 12 weeks

3.1.3. Comparison of new bone area ratios in control and experimental groups

3.1.3.1 Comparison of cases without bone graft

In Control and UV groups, new bone area ratio was measured in the same ROI which had been set up to measure the BIC ratio. The new bone area ratio was calculated as a percentage of the area occupied by new bone in ROI (Fig. 17). The mean value and standard deviation of the measurements were calculated and the Wilcoxon rank sum test was performed using these data, yielding values of 32.00 in group Control at 4 weeks, 40.62 in group UV at 4 weeks, 48.53 in group Control at 12 weeks, and 55.49 in group UV at 12 weeks. There was no significant difference between the two groups (Table. 6 & Fig. 18).

A. B.



C. D.

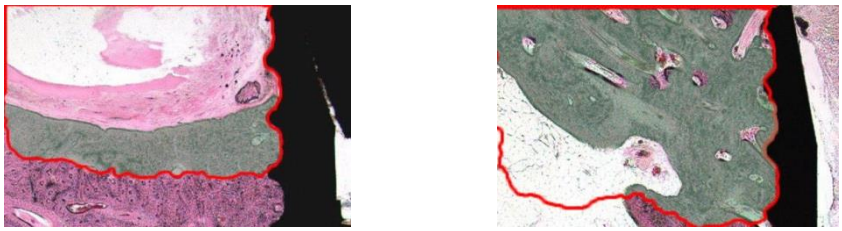


Fig.17 The representative of each group at 4 and 12 weeks.
(H&E stain, Original magnification: x12.5)
Green zone → New bone area, Red box → ROI
A: Control 4, B: UV 4, C: Control 12, D: UV 12

Table.6 The mean of new bone area ratios at 4 and 12 weeks.

Group		New Bone area ratio	
		4 wks	12 wks
Control	Mean	32.00	48.53
	SD	16.26	13.61
	SE	6.64	5.55
UV	Mean	40.62	55.49
	SD	39.33	29.57
	SE	27.81	20.91
[§]p-value		0.739	0.739

[§] The p-values were calculated using the Wilcoxon rank sum test.

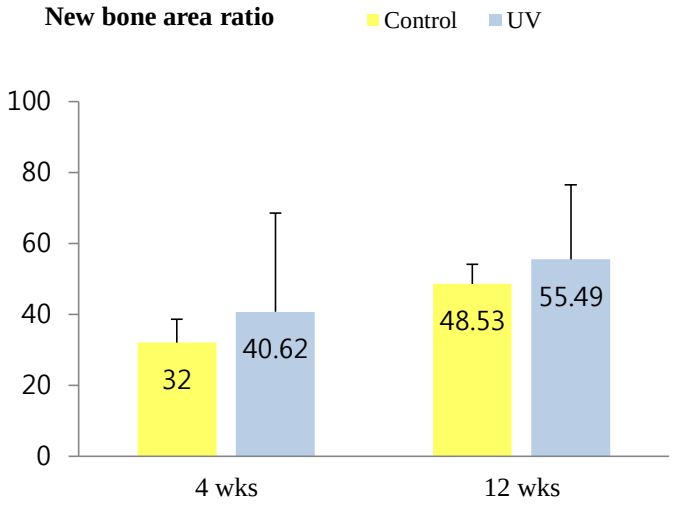


Fig.18 The mean of new bone area ratios at 4 and 12 weeks.

3.1.3.2 Comparison of cases with bone graft

In groups BG and UV/BG, the new bone area ratio was measured using the same methods described earlier (Fig. 19). The mean value and standard deviation of the

measurements were calculated and the Wilcoxon rank sum test was performed using these data, yielding values of 7.43 in group BG at 4 weeks, 15.09 in group UV/BG at 4 weeks, 14.20 in group BG at 12 weeks, and 5.28 in group UV/BG at 12 weeks. There was no significant difference between the two groups (Table. 7 & Fig. 20).

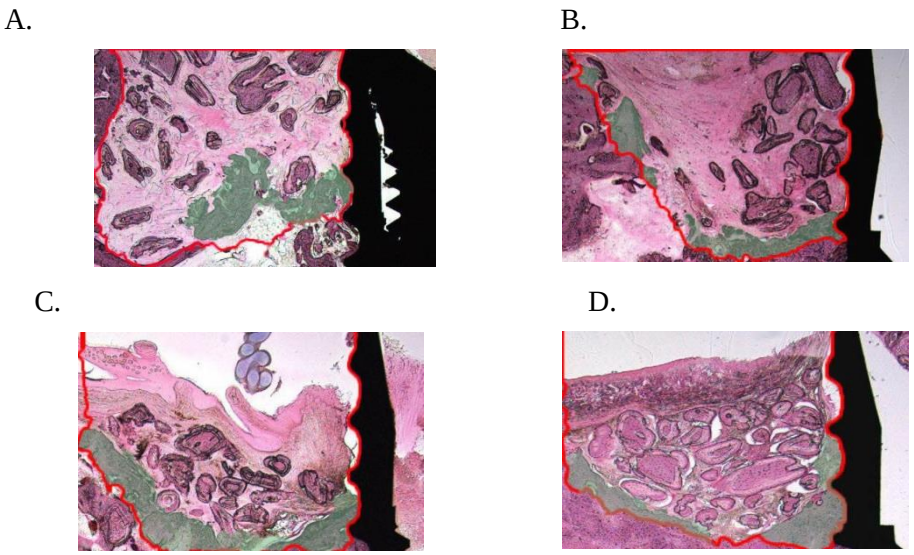


Fig.19 The representative of each group at 4 and 12 weeks.
(H&E stain, Original magnification: x12.5)
Green zone → New bone area, Red box → ROI
A: BG 4, B: UV/BG 4, C: BG 12, D: UV/BG 12

Table.7 The mean of new bone area ratios at 4 and 12 weeks.

Group		NB area ratio	
		4 wks	**12 wks**
BG	Mean	7.43	14.20
	SD	6.17	15.740
	SE	3.08	7.87
UV/BG	Mean	15.09	5.28
	SD	8.81	3.2
	SE	4.40	1.6
[§]p-value		0.149	0.386

[§] The p-values were calculated using the Wilcoxon rank sum test.

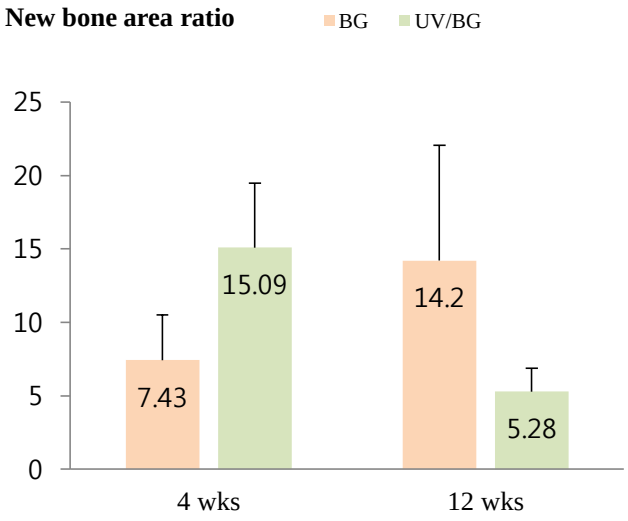


Fig.20 The mean of new bone area ratios at 4 and 12 weeks.

3.1.4. Comparison of remaining graft material area ratios in control and experimental groups

The remaining graft material area ratio was calculated as a percentage of the area occupied by remaining graft materials in ROI (Fig. 21). The mean value and standard deviation of the measurements were calculated and the Wilcoxon rank sum test was performed using these data, yielding values of 9.78 in group BG at 4 weeks, 21.40 in group UV/BG at 4 weeks, 12.08 in group BG at 12 weeks, and 16.48 in group UV/BG at 12 weeks. There was no significant difference between the two groups (Table. 8 & Fig. 22).

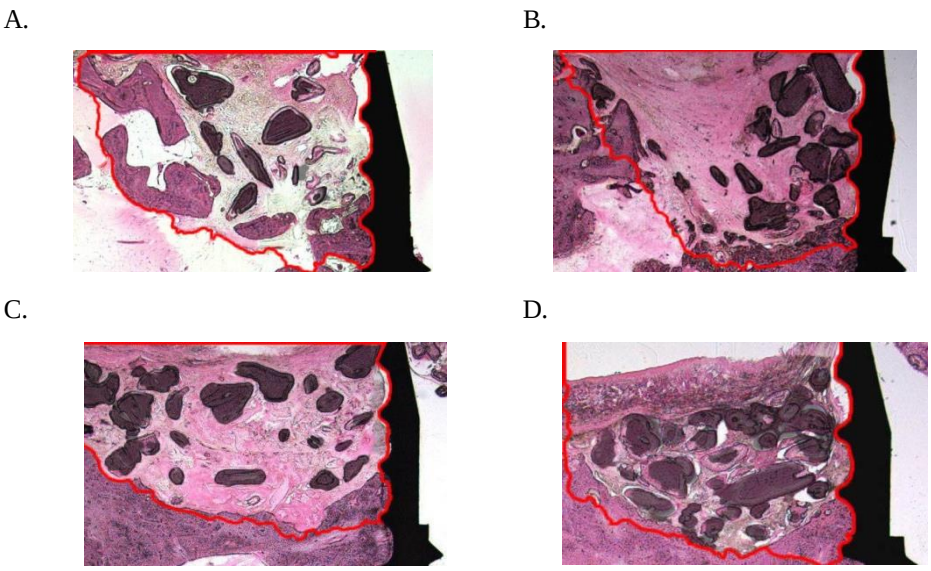


Fig.21 The representative of each group at 4 and 12 weeks.
(H&E stain, Original magnification: x12.5)
Gray zone → Graft material area, Red box → ROI
A: BG 4, B: UV/BG 4, C: BG 12, D: UV/BG 12

Table.8 The mean of remaining graft material area ratios at 4 and 12 weeks

Group		Remained graft material area ratio	
		4wks	**12wks**
BG	Mean	9.78	12.08
	SD	5.82	9.57
	SE	2.91	4.78
UV/BG	Mean	21.4	16.48
	SD	6.62	6.31
	SE	3.31	3.16
[§]p-value		0.057	0.386

[§] The p-values were calculated using the Wilcoxon rank sum test.

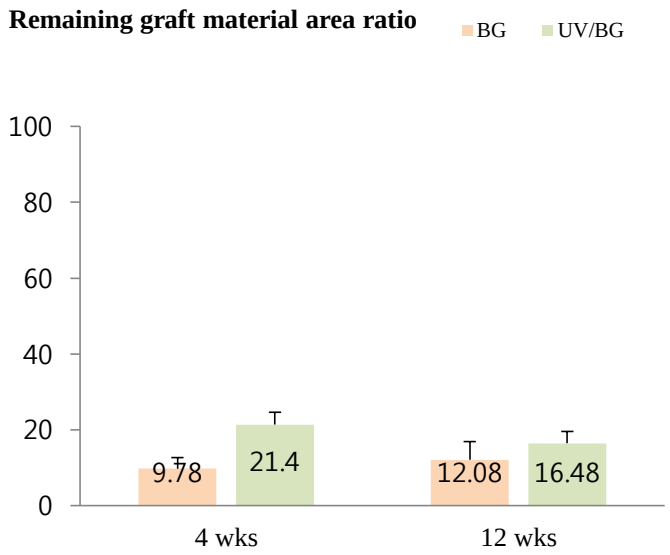


Fig.22 The mean of remaining graft material area ratios at 4 and 12 weeks.

3.1.5. Comparison of resorption area ratio between control group and experimental group

3.1.5.1 Comparison of cases without bone graft

In groups Control and UV, the resorption area ratio was measured in the same ROI which had been set up to measure the BIC ratio. The resorption area ratio was obtained as a percentage of the area in which bone filling did not occur in ROI (Fig. 23). The mean value and standard deviation of the measurements were calculated and the Wilcoxon rank sum test was performed using these data, yielding values of 36.13 in group Control at 4 weeks, 33.76 in group UV at 4 weeks, 46.09 in group Control at 12 weeks, and 12.15 in group UV at 12 weeks. There was no significant difference between the two groups (Table. 9 & Fig. 24).

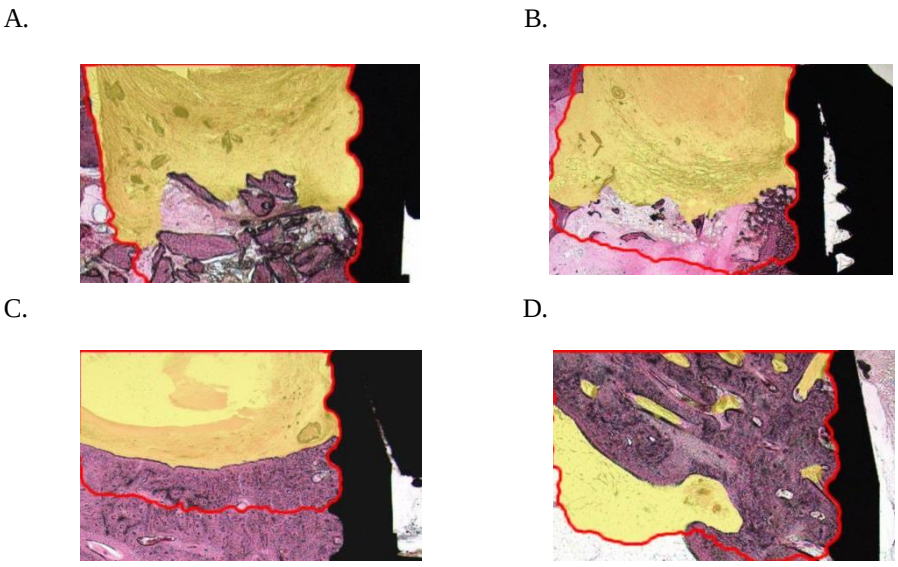


Fig.23 The representative of each group at 4 and 12 weeks.
(H&E stain, Original magnification: x12.5)
Yellow zone → Resorption area, Red box → ROI
A: Control 4, B: UV 4, C: Control 12, D: UV 12

Table.9 The mean of resorption area ratios at 4 and 12 weeks.

Group		Resorption area ratio	
		4 wks	12 wks
Control	Mean	36.13	46.09
	SD	7.91	17.99
	SE	3.23	7.34
UV	Mean	33.76	12.15
	SD	28.58	17.19
	SE	20.21	12.15
[§]p-value		1.000	0.096

[§] The p-values were calculated using the Wilcoxon rank sum test.

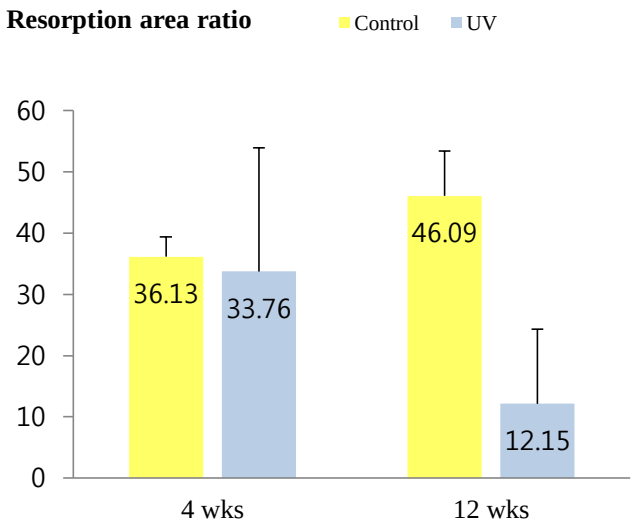


Fig.24 The mean of real resorption area ratios at 4 and 12 weeks.

3.1.5.2 Comparison of cases with bone graft

In groups BG and UV/BG, the resorption area ratio was measured using the same

methods described earlier (Fig. 25). The mean value and standard deviation of the measurements were calculated and the Wilcoxon rank sum test was performed using these data, yielding values of 17.40 in group BG at 4 weeks, 5.09 in group UV/BG at 4 weeks, 39.23 in group BG at 12 weeks, and 21.79 in group UV/BG at 12 weeks. There was no significant difference between the two groups (Table. 10 & Fig. 26).

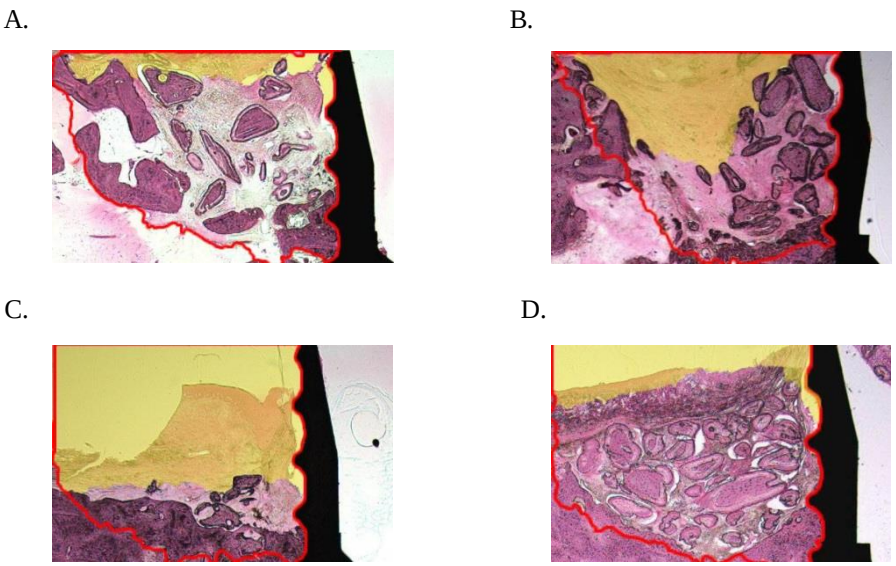


Fig.25 The representative of each group at 4 and 12 weeks.
(H&E stain, Original magnification: x12.5)
Yellow zone → Resorption area, Red box → ROI
A: BG 4, B: UV/BG 4, C: BG 12, D: UV/BG 12

Table.10 The mean of resorption area ratios at 4 and 12 weeks.

Group		Resorption area ratio	
		4 wks	12 wks
BG	Mean	17.4	39.23
	SD	19.3	35.91
	SE	9.65	17.96
UV/BG	Mean	5.09	21.79
	SD	10.18	17.66
	SE	5.09	8.83
[§]**p-value**		0.166	0.386

[§] The p-values were calculated using the Wilcoxon rank sum test.

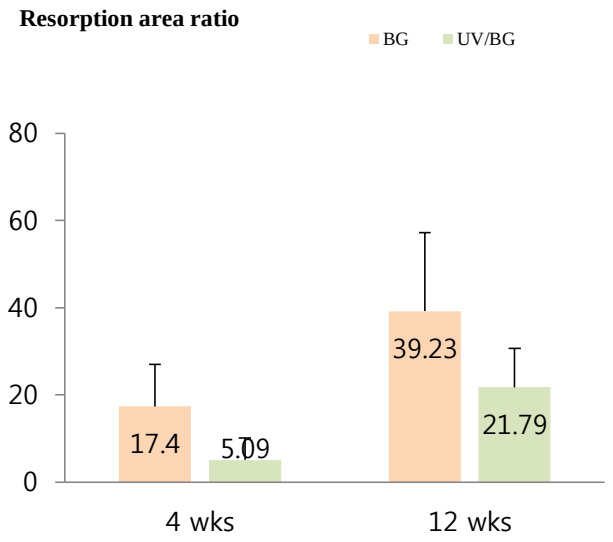


Fig.26 The mean of resorption area ratios at 4 and 12 weeks.

3.2. Micro-computed tomographic findings

3.2.1 Comparison of cases without bone graft

The amounts of new bone formed around the implant placed over a large critical defect were measured three dimensionally using a micro-CT scanner. In groups Control and UV, micro-CT bone volume ratio was measured in ROI, which had been newly set up for radiological analysis (Fig. 27). The measurements of the same region in the opposite side , used as the inner control site, were used to revise those in ROI. The relative values of each group were obtained by dividing the measurements in ROI by the measurements in the inner control site ROI. The mean value and standard deviation of relative values were then calculated. The Wilcoxon rank sum test was performed using these data, yielding values of 0.36 in group Control at 4 weeks, 0.52 in group UV at 4 weeks, 0.65 in group Control at 12 weeks, and 1.31 in group UV at 12 weeks. There was a significant difference between the two groups at 12 weeks (Table. 11 & Fig. 28).

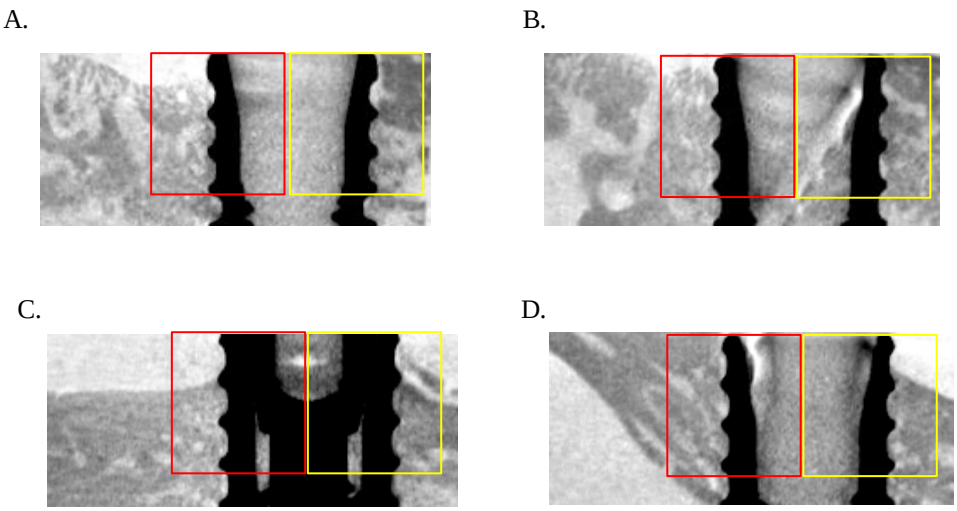


Fig.27 The representative of each group at 4 and 12 weeks (micro-CT images).

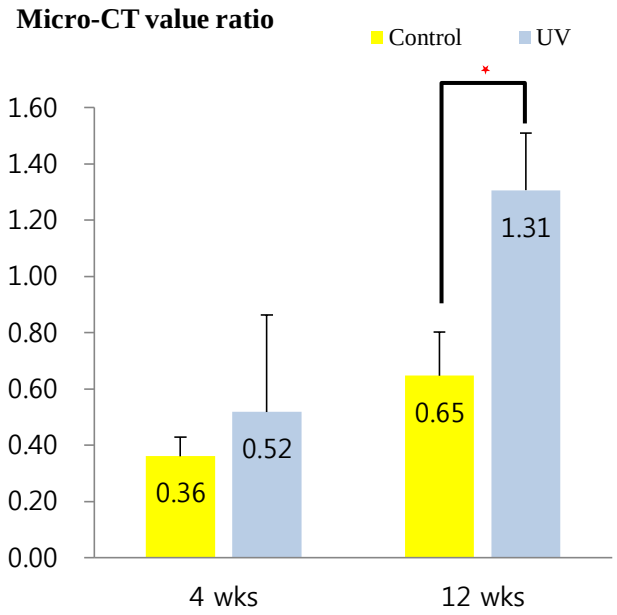
Red box → ROI in experimental site, Yellow box → ROI in opposite side

A: Control 4, B: UV 4, C: : Control 12, D: UV 12

Table. 11 The mean of micro-CT value ratios at 4 and 12 weeks.

Group		Micro CT value ratio	
		4 wks	**12 wks**
Control	Mean	0.36	0.65
	SD	0.17	0.38
	SE	0.07	0.16
UV	Mean	0.52	1.31
	SD	0.49	0.29
	SE	0.34	0.20
	[§]**p-value**	0.739	**0.046†**

[§] The p-values were calculated using the Wilcoxon rank sum test.
†:Statistically significant difference between groups (P<0.05)



* Red star indicate that there was significant difference (p<0.05).

Fig.28 The mean of micro-CT value ratios at 4 and 12 weeks.

3.2.2 Comparison of cases with bone graft

In groups BG and UV/BG, micro-CT bone volume ratio was measured using the same methods described earlier (Fig. 29). The relative values (micro-CT value ratio) of each group were obtained and the mean value and standard deviation of relative values then calculated. The Wilcoxon rank sum test was performed using these data, yielding values of 0.28 in group BG at 4 weeks, 0.46 in group UV/BG at 4 weeks, 0.53 in group BG at 12 weeks, and 0.55 in group UV/BG at 12 weeks. There was a significant difference between the two groups at 4 weeks (Table. 12 & Fig. 30).

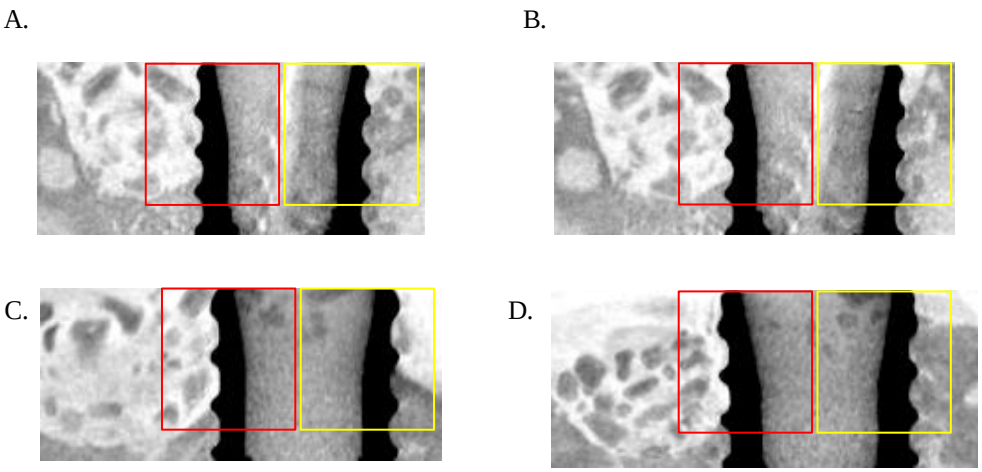
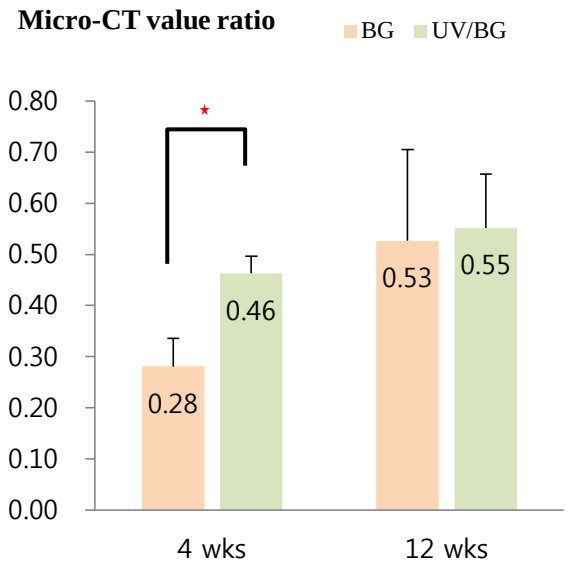


Fig.29 The representative of each group at 4 and 12 weeks (micro-CT images).
Red box → ROI in experimental site, Yellow box → ROI in opposite side
A: BG 4, B: UV/BG 4, C: BG 12, D: UV/BG 12

Table. 12 The mean of micro-CT value ratios at 4 and 12 weeks.

Group		Micro CT value ratio	
		4 wks	**12 wks**
BG	Mean	0.28	0.53
	SD	0.11	0.36
	SE	0.05	0.18
UV/BG	Mean	0.46	0.55
	SD	0.07	0.21
	SE	0.03	0.11
[§]**p-value**		**0.021†**	0.773

[§] The p-values were calculated using the Wilcoxon rank sum test.
†:Statistically significant difference between groups (P<0.05)



* Red star indicate that there was significant difference (p<0.05)

Fig.30 The mean of micro-CT value ratios at 4 and 12 weeks.

IV. Discussion

For implant surgeries with large critical defects, placements with a bone graft are known as a general treatment method. It was found that grafting materials were eventually absorbed as the gap was filled with new bone and that in the long term, the rates of osseointegration and osteogenesis were higher.[17,18] One study also showed the positive effect of bone graft in maintaining the volume of defect, thus leading to long-term success of the implant.[22] The 2012 case report of Snyder,[13] observed long-term clinical success for implant placements with a bone graft over bone defect areas.

However, previous research reported that both early proliferation of osteoblast and new bone formation development were hindered by graft material.[14-16] It was assumed that the success rate for implant placements with a bone graft could be even higher if influences of graft materials were partially offset through the BIC increase of surface. Therefore, experiments with UV photofunctionalization were performed on implant surfaces based on the research results of Ogawa et al., which showed that osseointegration and osteogenesis became more advantageous at an early placement stage with UV photofunctionalization on surfaces due to higher BIC, increased hydrophilicity and the enhanced electrostatic status of implant surfaces.[1,19] Experimental difficulties were found after this experiment was performed with female beagle dogs. These difficulties were due to the female beagle dog having a lower amount of bone, a relatively smaller mandible, and hormonal changes that affected the healing process in bone defect.[23,24] The experiment results did not show expected levels of healing. Among samples for each

group, there existed some with noticeably low result values. Such cases would not have had much influence on overall experiment results (even with remarkably low result values) if there had been more testing samples. In addition, if implants had been placed at all the same positions due to many sample sizes, the differences in results according to placement locations could be eliminated. It is expected that subsequent research would yield more meaningful experimental results if experiments were performed with dogs having sufficient bone and a large mandible.

This study evaluated the effects on osseointegration and bone formation of UV photofunctionalization on SA surface implants as successfully used in clinics. For bone graft material, demineralized freeze-dried bone allograft (DFDBA) was used. This material is known to increase the ability of osteoinduction due to its bone inductive protein, exposed by demineralizing the cortical bone with hydrochloric acid.[25] The rate of its resorption, however, is hastened due to its demineralization. Therefore, the level of osteoinduction which provides proper scaffold for new bone formation, is lower than that of Freeze Dried Bone Allograft (FDBA) or xenograft.[26,27] A study by Vicente et al. in 2006[20] found no statistically significant difference between DFDBA and demineralized bovine bone groups in terms of bone formation and BIC after grafting over defects. DFDBA was, thus, applied in this study in spite of being an experiment with dogs. For this reason, it was expected that the effect of DFDBA on osteoinduction would be insufficient compared with use in human. Also, in this experiment, the membrane was not applied after bone graft due to a previous study[22] showing that periosteal preservation appeared critical in maintaining long-term, stable bone volume in a large defect site.

A large critical bone defect 5 mm width was designed for the present study. The term "Critical Size Defect" (CSD) was originally defined by Schmitz & Hollinger -as "the smallest size intra-osseous wound in a particular bone and species that will not heal spontaneously during the lifetime of the animal."[28] However, in recent years, the meaning has changed to "the critical-size defect in animal research refers to the size of a defect that will not heal over the duration of the study."[29] The opposite side of the experimental site (the inner control site) was also set up to compensate for the effect of implant placement position. The measurements in ROI of the experimental site were revised based on the measurements in ROI of the inner control site. This allowed clearer evaluations of UV photofunctionalization effects.

Urist reported that[30] when grafting DFDBA, new bone deposition was found within two weeks, the amount of new bone formation increasing after a few weeks. Melloning et al. [31] also found that new bone formation increased considerably with the use of DFDBA. Another study reported that new bone formation was promoted three months after grafting.[32] The study of Hur et al. using DFDBA[33] reported that graft material was actually reduced after eight weeks and that new bone formation was clearly increased. Therefore, measurements were done at four weeks, when new bone formation is increased compared to the beginning of grafting, and at twelve weeks, when graft material decreases to a certain point and new bone visibly increases.

In the experiment, two groups were established, with and without bone grafts, and then further divided into groups with UV photofunctionalization and (control) groups without it. First, effects of UV photofunctionalization on implant surfaces and bone defect were

examined for bone defects without bone graft. Subsequently, these UV photofunctionalization effects were compared with those for bone defect with bone graft. Since previous studies proved the effect of UV photofunctionalization on implant placements without a bone graft,[1,6,7,19,34] this study was conducted using a reduced sample size of UV photofunctionalized implants without bone graft.

First, for inner control groups opposite the bone defect sites, measurements of Bone-to-Implant Contact (BIC) were compared according to UV photofunctionalization. Compared with mean values, BIC values of implant surfaces with UV photofunctionalization were higher than control groups. However, there was no statistically significant difference between experimental groups and control groups. In 2009, Aita et al.[5] reported significantly higher BIC values of implants with UV photofunctionalization than in implants without it in rat experiments. Another experiment using dogs yielded similar results.[35] However, there was no statistically significant difference in this experiment even though implants with UV photofunctionalization showed relatively higher mean values. For this reason, the limitation of the experiment was thought to be the small numbers of samples. Moreover BIC values at twelve weeks were found to be 20% lower than ones at four weeks, perhaps because the natural healing around implants did not occur over time since the experiment model was a female beagle with a small amount of bone and small mandible. Another limitation was the lack of sufficient bone formation to present clear effects of UV photofunctionalization.

Next, measurements for Bone-to-Implant Contact (BIC) in ROI of each group were compared across the board. BIC in both ROI of the experimental site with bone defect

and ROI of the inner control site were measured in each group. The locations of the implant placement in each sample varied and not all samples were done on a single experimental object. For this reason, the BIC in ROI of the experimental site of two samples were not actually the same when the BIC in ROI of the inner control site was different even if samples had the same BIC in ROI of the experimental site. The revised BIC ratios for each sample were obtained by dividing the ROI measurements by those of the control site and comparing them. The higher revised the BIC ratio, the higher the BIC on the implant surface with better subsequent osseointegration. The groups of implants placed over bone defect without graft, yielded similar BIC ratios regardless of the presence of UV treatment. There were minor positive effects of osseointegration on implant surfaces in these groups. Furthermore, BIC ratios were increased in the twelve-week-groups compared with the four-week-groups, unlike the reduced patterns of BIC ratios over time in inner control sites. Since the decrease in BIC of the inner control site was greater than at the experimental site, BIC ratios calculated as the ratio of the two measurements increased. In the group of implants placed over bone defect with graft, BIC ratios of UV-treated implant surfaces were higher than those at four weeks but lower than those at twelve weeks. There was no statistically significant difference between groups. However, as each sample could have highly reliable results per the characteristics of animal experiments, UV photofunctionalization was thought to have improved BIC of implant surfaces despite negative effects of graft material on osseointegration at the early stage. As BIC values of experimental sites from the UV/BG group at four weeks were actually more than twice the BIC values for experimental site in the GB group, relative BIC values were higher in the UV/BG group than in the BG group. On the other hand,

experimental site BIC values in the UV/BG group at twelve weeks showed little difference compared to experimental site BIC values in the BG group. BIC measurements for the control site showed a large difference and thus relative BIC measurements were higher in BG groups than in the UV/BG group. This suggests that BIC of implant surfaces increases by UV photofunctionalization for bone defects with a bone graft at the initial stage of the placement. The decrease in experimental site BIC values at twelve weeks could be due to characteristics of female beagles and to weak bone healing caused by dissipation of graft material. To resolve these issues in further research, it may be necessary to use male dogs with high amount of bone and membrane to prevent such dissipation.

Next, the area of new bone development in the ROI was measured put into a ratio with the whole area of the ROI. The mean of groups with UV photofunctionalization was slightly higher at both four and twelve weeks when the implant was placed without a bone graft, yet with no statistically significant difference. As UV photofunctionalization affected the bone defect area itself, more new bone might have arisen. In the future, the effects of UV photofunctionalization can be more clearly verified by separately removing and measuring the amount of bone on the implant surface. The UV/BG group at four weeks had higher values than the BG group. The UV photofunctionalization had a positive influence on early new bone formation for bone defect with a bone graft. However, BG group measurements were higher than in the UV/BG groups just as results with the BIC ratio at twelve weeks. Likewise, external factors may have slowed bone healing gradually.

The area of graft materials remaining in ROI was measured for comparisons. The higher the remaining graft material ratio, the more graft material remained in the defect area and thus the greater the subsequent volume of maintained defect. The beagle dogs were grafter with DFDBA, which is more effective in osteoinduction than in osteoconduction.[25-27] However, it was expected that it would work as a scaffold for maintaining the volume of defect rather than as an osteoinducer because human bone was grafted on dogs. Because the grafted demineralized materials were absorbed rapidly, there were questions whether they could serve as a scaffold for a long time. In fact, group BG showed higher measurements at 4 weeks than at 12 weeks. Group UV/BG showed less remaining grafting material at 12 weeks. Based on comparisons of the actual measurements of each group, values were inconsistent and had large standard deviations at 12 weeks. It is important in a one-wall defect to prevent dissipation of graft materials.[36,37] In the present study, however, no membrane or any other methods for maintenance of graft material were used, with possible implications for remaining graft materials. Subsequent experiments may yield more reliable results if methods for graft material retention are used.

The resorption area in the ROI was obtained by measuring the area excluding the part in which the height of the defect was maintained due to new bone and graft materials, the resorption area ratio being that of the measured area to the area of the entire ROI. This ratio reflected the degree of defect volume maintenance. In the group without bone graft, UV treatment showed little effect at 4 weeks. With time, the resorption area ratio decreased in the UV groups. The smaller the resorption area ratio, the greater the volume

of maintained defect. This may result from the large amount of new bone or remaining graft materials as well as from less resorption of bone itself. The difference in resorption rates was due to the smaller resorption area ratio in group UV at 12 weeks, a consequence of the posterior implant placements. There were large differences in resorption area ratios between groups Control and UV at 12 weeks as opposed to the similarity in new bone area ratio observed by histomorphometrical analysis. The differences in bone volume measured using micro-CT surely account for the differences in resorption area ratio. Whereas the resorption area was measured in one plane with light microscopy, the amount of new bone was measured in three dimensions with a micro-CT scanner. These differences must be considered in calculating each measurement and may underlie limitations in calculating the resorption area using micro-CT measurements. For this reason, the implant placement position was considered the major cause of differences in resorption area ratio at 12 weeks. Subsequent experiments should employ more subdivided positions for implant placement to enable greater uniformity of placement. In the bone graft groups, the resorption area ratio in group UV/BG was less than in group BG at 4 weeks. This may be attributed to the greater amount of new bone and remaininggraft material in group UV/BG than in group BG. Similarly, at 4 weeks, the resorption area ratio in group UV/BG was less than in group BG at 12 weeks, a consequence of the difference in resorption rates due to posterior implant placement. Few differences in new bone area ratios between groups UV/BG and BG were observed using the micro-CT scanner and the new bone area ratio of group UV/BG was less than that of group BG in histomorphometrical analysis. Due to the difference in resorption rate caused by implant placement position, the amount of remaining graft material in the UV/BG

group exceeded that of group BG. This may explain the relatively reduced resorption.

Lastly, the amount of bone in the ROI was measured and compared using a micro-CT scanner. Micro-computed tomography has been used extensively to characterize bone tissue and has the potential to overcome various limitations.[38] Studies by Van Oosterwyck et al.[39] and Frank Butz et al.[40] proved the effectiveness of micro-CT in quantitative and qualitative analysis of peri-implant. The percent of bone volume values and the percentile ratio of radio-opaque regions of interest over total defect volume were measured in three dimensions.[41] The new ROI was set up in a rectangular parallelepiped 3.5 mm in width (mesio-distal) from centers of implants, 3.0 mm in length (bucco-lingual), and 3.0 mm in height, and the amount of bone was measured in this area. This amount of bone formed around the implant, rather than the amount of bone formed in entire defect, has been reported to indicate the degree of osseointegration.[42] The new ROI was set up for measuring the amount of new bone around the implant and the measurements were obtained in this new ROI. Just as in measuring the BIC, the revised micro-CT values ratio of each sample were obtained by dividing the measurements in the new ROI by those in the new ROI of inner control site and compared. The higher the revised micro-CT value ratio, the greater the subsequent new bone formation. In the group without bone graft, similar patterns in histomorphological measurements at 4 weeks appeared, whereas the UV group measurements were about two times higher than the control group ones. There was a statistically significant difference. These distinctions were due to differences between measurement with light microscopy and micro-CT scanner. The amount of new bone was measured in three dimensions by the micro-CT scanner, but in only one plane

through light microscopy. The variation in measurements of new bone growth may be due to inherent differences among the ROI. If the ROI of these measuring methods are equivalent in subsequent experiments, it will be possible to evaluate the effects of UV photofunctionalization on implant surfaces more precisely. In the bone graft groups, group UV/BG scored higher in new bone volume measurements than did group BG at an early stage (4 weeks). This agrees with the pattern of new bone formation measurements through ight microscopy. Based on these results, it was concluded that UV photofunctionalization had a positive effect on new bone formation of bone defect with bone graft. On the other hand, the results of each group in late stage (12 weeks) were not significantly correlated per the presence of UV treatment. There were few differences between groups in the amount of new bone area in histomorphometrical measurements whereas the measured value of group UV/BG was greater than that of group BG. The result of these two measurement methods were quite inconsistent due to the micro-CT scanner's measurement of volume in three dimensions. There were otherwise no statistically significant differences between the two measurement methods. Therefore it was difficult to evaluate the effect of UV photofunctionalization effect on new bone formation at 12 weeks. Better osseointegration and greater osteogenesis was shown on UV-treated implant surfaces than on SA surface implants placed over large critical defects with bone graft in the early stage. A statistically significant difference was confirmed using micro-CT. However, UV photofunctionalization barely showed any effect on implant surfaces placed over the critical defect with bone graft over time. The results of these experiments do not completely rule out the effect of implant location. Subsequent experiments must maintain uniform implant conditions as much as possible, including

placement location, by increasing the number of samples and by subdividing the location of implant placement. If male dogs with large jaws and abundant bone are used in future experiments, results will be significantly different. Furthermore, if membranes are used after bone graft to prevent graft materials from dissipating,[36,37] it will be possible to evaluate UV photofunctionalization effects on bone defect with bone graft more clearly per time period.

V. Conclusion

The purpose of this study was to compare and evaluate effects and influences of UV photofunctionalization on an implant with bone graft next to an artificially created critical one wall defect that would not heal over a certain period of time. In spite of experimental limitations and difficulties, the following results were obtained:

1. In both the 4 and 12-week groups, UV-treated implant surfaces achieved better osseointergration than did SA implant surfaces, while both implant surfaces had direct contact with bone.

2. UV photofunctionalization barely showed any effect on implant surfaces placed over the critical defect without bone graft. After 12 weeks, however, UV photofunctionalization led to increased new bone formation.

3. In implant surfaces placed over the critical defect with bone graft, UV photofunctionalization increased BIC and new bone formation at the initial stage (4 wks.)

4. It was difficult to evaluate the effect of UV photofunctionalization on each condition at 12 weeks due to difficulties with the experimental model such as mandibular dimension, the quality and quantity of alveolar ridge at the surgical site, and the use of female beagle dogs.

Based on the results of this study, UV photofunctionalization on the surface of

implants next to large critical defects with bone graft may increase initial osseointegration and osteogenesis. If the limitations of this study are addressed in subsequent experiments, it will be possible to examine UV photofunctionalization's positive effects on implants placed over large critical defects with bone graft over the long term.

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국문요약

UV 조사처리가 골이식이 동반된 1벽성 골결손부위에 식립한 임플란트에 미치는 영향

: 비글 견을 이용한 예비연구

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목적: 임플란트 표면에 대한 UV 조사 처리는 임플란트 표면의 성질을 생리활성으로 변화시킴으로써 임플란트 주변에서의 골 유착과 골 생성에 긍정적인 영향을 미친다는 것이 알려져 있다. 이 연구의 목적은 골 이식 동반여부에 따른 골 결손부위에 UV 조사 처리 임플란트를 식립한 후 이를 조직계측학적 및 방사선 학적으로 비교 평가해보는 것이다.

방법: 4 마리의 암컷 비글 견을 골 이식 유무에 따라 두 그룹으로 나눈 후, 각각의 그룹을 다시 UV 조사 처리 유무에 따라 실험군과 대조군으로 분류하였다. 하악의 소구치를 발치하고 12 주 후에 각각의 그룹별 조건에 따라 임플란트를 식립 하였다. 왼쪽과 오른쪽, 각각 4 주와 12 주의 치유 기간이 경과한 후에 희생하였고, 조직계측학적 측정과 방사선학적 측정을 위한 시편을 제작하였다.

결과: 4 주군과 12 주군 모두에서 골과 직접 접촉해 있는 임플란트 표면의 골 유착은 UV 조사 처리한 임플란트의 표면에서 더 많이 이루어졌다. 골 이식을 동반하지 않은 골 결손부위에 식립한 임플란트 에서는 UV 조사 처리의 영향이 크게 나타나지 않았다. 12 주가 지난 후에는, 신생 골의 형성을 증가시키는 양상을 보였다. 골 이식이 동반된 큰 골 결손부위에 식립한 임플란트 에서 UV 조사 처리는 초기(4 주)의 BIC 와 신생 골 생성을 증가시키는 긍정적인 영향을 미쳤다.

결론: 골 이식을 동반하는 골 결손부위에 임플란트를 식립할 때, 임플란트 표면에 UV 조사 처리를 하는 것이 초기의 골 유착과 골 생성에 있어서는 유리할 것으로 생각된다. 추후의 실험에서 이 실험의 한계점들을 보완한다면, 장기적으로도 골 이식이 동반된 골 결손부위에 식립한 임플란트의 성공에 UV 조사 처리가 긍정적인 영향을 미치는가에 대해 확인해 볼 수 있을 것이다

핵심 되는 말: UV 조사처리, 골 이식, 골 결손 부위, 임플란트, BIC