The influence of perforating the autogenous block bone and the recipient bed in dogs.
: a radiographic analysis

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The influence of perforating the autogenous block bone and the recipient bed in dogs. : a radiographic analysis

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그동안 치주과 대학원을 통해 새로운 지식을 접하고 개원생활에 소중한 활력을 느껴온지도 어느덧 5 년의 세월이 흘렀습니다. 졸업이후 10 년만에 다시 학교의 품으로 돌아와 수업을 듣고 논문을 준비하면서 치과의사로서의 삶에 있어 필요한 기본적인 자세를 되짚어보게 되는 기회가 되었습니다.

이번 논문이 결실을 맺기까지 큰 도움을 주신 많은 분들께 머리숙여 깊이 감사드립니다. 항상 온화한 미소로 따뜻하게 감싸주시고 대학원 기간내내 든든한 버팀목이 되어주신 채중규 지도교수님, 동물실험을 비롯하여 논문전반에 걸쳐 세심한 지도편달을 해주시고 SCI 저널에 채택되는 영광까지 얻도록 도와주신 정의원 교수님, 논문의 조직학 파트를 맡아서 함께 고민하고 세부적인 과정까지 힘써주신 차재국 선생님, CT 를 이용한 삼차원적 분석을 도와주신 정은선 전 연구원님 모두 진심으로 감사드립니다.

아울러 치주학에 대한 창의적인 생각을 바탕으로 끊임없이 도전하고 탐구하는 모습을 보여주시며 이끌어주신 김종관 교수님, 조규성 교수님,

최성호 교수님, 김창성 교수님을 비롯하여 전체 치주과 수련의 선생님들께도 감사의 말을 올리고 싶습니다.

마지막으로, 성실한 삶의 모습을 가르쳐주셨던 하늘에 계신 아버님, 늘 걱정해주시고 챙겨주시는 어머님, 용기를 북돋아 주시고 물심양면으로 도와주신 장인 장모님, 부족한 나를 채워주는 사랑하는 아내 주원이와 두딸 유진, 유나에게 사랑한다는 말을 전하고 싶습니다.

이번 대학원 생활을 계기로 더욱더 분발하여 환자의 고통을 좀더 이해하고 근거에 바탕을 둔 치료를 지향하며 새로운 지식을 추구하는 치과의사가 되도록 노력하겠습니다.

감사합니다.

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Abstract

The influence of perforating the autogenous block bone and the recipient bed in dogs. : a radiographic analysis

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(Directed by Professor Jung-Kiu Chai)

Objectives: This study evaluated radiographically the integration and volume maintenance of grafted autogenous block bone under various cortical bone perforation conditions in dogs.

Material and methods: Five mongrel dogs were used. Each dog received four differently prepared onlay block bone grafts: a solid block graft was fixed on either (1) a cortically perforated recipient bed (SGPR) or (2) a nonperforated recipient bed (SGNPR), a perforated block graft was fixed on either (3) a nonperforated recipient bed (PGNPR) or (4) a cortically perforated recipient bed (PGPR). The animals were sacrificed at 1 day, 4 days, 10 days, 4 weeks, and 8 weeks after surgery. Specimens were prepared and radiographic analysis was conducted by using micro-computed tomography. The residual bone volume (RBV; mm^3), cross-sectional bone area (BA; mm^2), and residual height (RH; %) of the grafted block bone were measured radiographically.

Results: The interface between the recipient bed and the graft showed no signs of bone integration at 1, 4, and 10 days of healing. However, at 4 weeks of healing, bone integration was observed in all groups. The RBV, BA, and RH of the grafts gradually decreased by 4 weeks of healing. At 8 weeks, the PGPR condition exhibited a higher RBV, BA, and RH than the other conditions, while the SGNPR condition exhibited the lowest RBV, BA, and RH.

Conclusion: Within the limitations of this study, it can be concluded that intentional cortical perforation on the recipient bed and block bone graft may influence volume maintenance of the graft.

Key words: alveolar ridge augmentation, bone regeneration, decortication, X-ray microtomography, dogs

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I . Introduction

The bone available for the placement of dental implants may be insufficient due to periodontitis, tooth extraction, trauma, infection, or the long-term use of removable prostheses (Lekovic et al., 1997; Ong et al., 2008). Most of the resorption process occurs during the first year after tooth extraction (Atwood, 1971). Ridge resorption may lead to an unfavorable maxilla–mandible relationship, and may aggravate the proximity to the maxillary sinus and the mandibular nerve (Misch et al., 1992).

From functional and esthetic points of view, severely resorbed alveolar bone requires a ridge augmentation procedure to achieve the appropriate width and height to enable successful placement of an implant. Various methods have been introduced to augment the volume of the resorbed bone, including guided bone regeneration (GBR), autogenous block bone (ABB) graft, distraction osteogenesis, and ridge splitting (McAllister & Haghighat, 2007).

In particular, the ABB graft approach has been the method of choice in unprotected areas that are subject to compressive forces such as mastication, due to its dimensional stability and resistance to deformation (Barbosa et al., 2009). Thus, a considerably predictable amount of horizontal augmentation on the narrow alveolar ridge can be gained (von Arx & Buser, 2006).

In spite of the potential osteogenic properties of autogenous bone grafts (Bauer & Muschler, 2000; Khan et al., 2005), a tendency toward unpredictable resorption and morbidity at the donor site represent obstacles to this procedure (Donos et al., 2005; Jardini et al., 2005). Concerning the resorption of the ABB, Bruggenkate et al. (1992)

reported that 50% of the bone graft volume was lost after 6 months in humans (ten Bruggenkate et al., 1992), and Widmark et al. (1997) observed that 1 year postoperatively, 60% of the lingual–facial thickness of the ABB harvested from the mandibular symphysis was lost.

Physiologic stress applied to the graft, embryological origin, and graft-to-recipient bone contact have been suggest as factors that affect resorption in bone graft healing (Kusiak et al., 1985; Alberius et al., 1996a; De Marco et al., 2005). Rigid graft fixation, recipient bed perforation, and the use of a membrane have been tested in attempts to fulfill these factors and minimize bone resorption in preclinical study (Adeyemo et al., 2008). During the healing process of the ABB graft, the necrotic bone is replaced with viable bone by creeping substitution (Burchardt & Enneking, 1978). Therefore, the angiogenesis and revascularization of the graft are crucial for integration (De Marco et al., 2005).

The dense architecture and lack of endosteal cells within the cortical bone may hamper proper revascularization. The process of perforating the cortical bone has been performed routinely to promote incorporation of the graft into the recipient site during the ABB graft and GBR procedures (Simion et al., 2007). Perforation of the cortical bone can provide a pathway for blood vessels and progenitor cells to approach from the endosteal compartment (Hämmerle et al., 1995; Schmid et al., 1997; Lundgren et al., 2000). In addition, it may strengthen the physical bond between the grafted bone and the recipient site (Alberius et al., 1996b; Gordh et al., 1997). Frost (1983) argued that a noxious stimulus encouraged the regional acceleratory phenomenon of the normal healing process in both hard and soft tissues. In this context, cortical perforation may be regarded as a noxious stimulus that promotes local healing. De Carvalho et al. (2000) reported that integration of the ABB graft occurred mainly in groups with perforated or decorticated recipient beds in dogs. Majzoub et al. (1999) also demonstrated that decortication of the calvaria promotes bone formation in rabbits.

It appears that decortication does not produce unfavorable healing effects, but it does tend to extend the operation time and jeopardize visibility in the operation field as a result of bleeding. Additional surgical trauma can also cause more loss of blood and bone substance, and has the potential to generate overheating via the drilling bur. Adeyemo et al. (2008) observed the healing process of onlay mandibular bone grafts in 12 sheep and reported that there were no significant differences between the perforated and nonperforated groups after an 8 week healing period. They asserted that rigid fixation and intimate contact between the graft and the host bed were more important factors than cortical bed perforation.

As mentioned above, the results of animal experiments have been controversial, and it is not clear whether the decortication process would be helpful or harmful to bone healing. The aim of this comparative study was to evaluate how various perforation conditions in either the ABB or the recipient bone bed influence the integration and volume maintenance of the grafted bones in a series of healing periods using micro computed tomography (micro-CT).

II . Material and methods

1. Experimental animals

Five male mongrel dogs weighing approximately 30 Kg each and aged about 12 months, were used. They had no systemic diseases and had healthy dentition and periodontal tissues. The selection and management of the experimental animals, and the surgical operations followed the routine procedure approved by the Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea (permission no. 07-041).

2. Assignment of experimental groups

All animals were given one of the following experimental treatments according to perforation conditions of the ABB or recipient bed:

● ***SGNPR*** group: a solid block graft was fixed onto a nonperforated recipient bed.

● ***SGPR*** group: a solid block graft was fixed onto a perforated recipient bed.

● ***PGNPR*** group: a perforated block graft was fixed onto a nonperforated recipient bed.

● ***PGPR*** group: a perforated block graft was fixed onto a perforated recipient bed.

All of the 4 conditions were randomly assigned to both sides of the maxilla and all were observed after various healing periods: 1, 4, and 10 days to evaluate the early healing pattern, and 4 and 8 weeks for the late healing pattern.

3. Surgical procedures

All surgical operations were performed under general anesthesia. The dogs were administered a preanesthetic intravascular injection of atropine (0.05 mg/kg; Kwangmyung Pharmaceutical, Seoul, Korea) and an intramuscular injection of xylazine (2 mg/kg; Rompun, Bayer Korea, Seoul, Korea) and ketamine hydrochloride (10 mg/kg; Ketalar, Yuhan, Seoul, Korea). Inhalation anesthesia was administered using 2% enflurane (Gerolan, Choongwae Pharmaceutical, Seoul, Korea). Infiltration anesthesia was administered using lidocaine (2% lidocaine hydrochloride–

epinephrine 1:100,000, Kwangmyung Pharmaceutical). The flap was set above the maxillary premolar area in preparation for the recipient bone bed. A horizontal incision was made 5 mm apically to the gingival margin of the premolar from the distal side of the third premolar to the mesial side of the first premolar, from where vertical incisions were extended apically (Fig. 1). Following elevation of the full-thickness flap, two circles for recipient beds were marked using a trephine bur of 8 mm diameter under copious saline irrigation.

Five cortical perforations were prepared within the circle using a 1.5-mm-diameter carbide bur for the SGPR and PGPR groups, while no perforation was prepared for the SGNPR and PGNPR groups (Fig. 2).

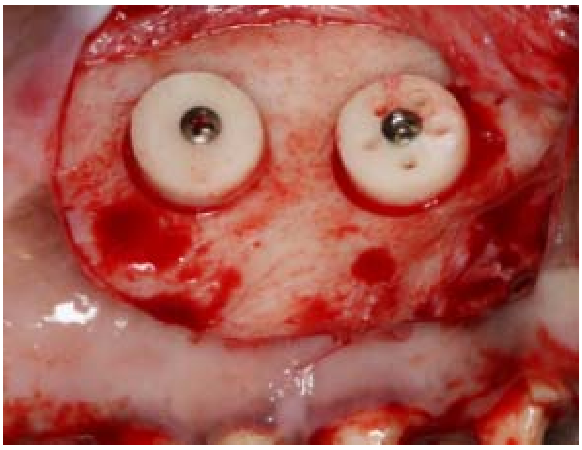


Fig. 1. Clinical photography showing the flap design.

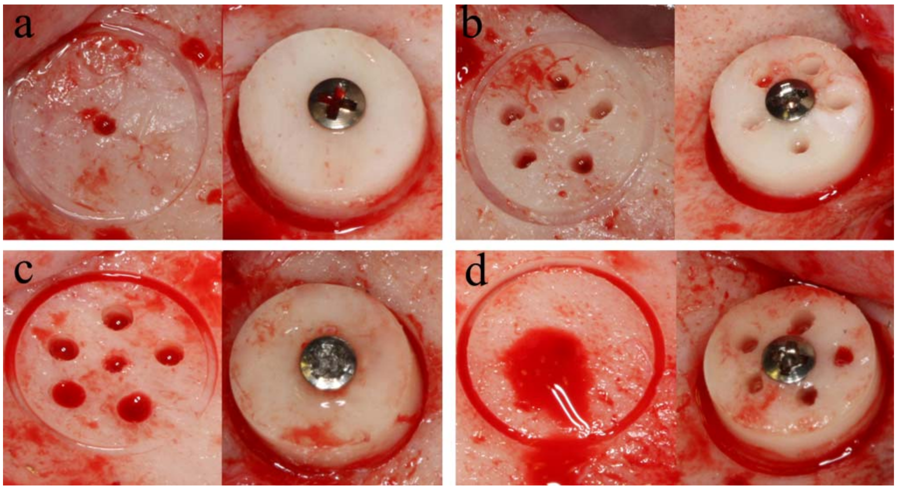


Fig. 2. Clinical photography showing the study design. (a) SGNPR group: the solid block graft was fixed onto a nonperforated recipient bed. (b) PGPR group: the perforated block graft was fixed onto a perforated recipient bed. (c) SGPR group: the solid block graft was fixed onto a perforated recipient bed. (d) PGNPR group: the perforated block graft was fixed onto a nonperforated recipient bed.

The full-thickness flap was elevated at the ascending ramus and buccal shelf area of the posterior mandible for preparation of the donor site, where four ABBs were obtained with a trephine bur of the same diameter. The collected block bones were trimmed to a thickness of 3 mm, and two of them were penetrated at five positions with a 1-mm-diameter carbide bur (PGNPR and PGPR conditions; Fig. 1). After making a central hole in all blocks for the fixation screw, each of the ABBs was intimately fixed onto the corresponding recipient bed using a fixation screw (Membrane screw, Dentium, Seoul, Korea). The flap was repositioned and sutured using a simple interrupted suture technique with 4-0 absorbable suture material (Monosyn 4.0 Glyconate Monofilament, B. Braun Tuttlingen, Germany).

4. Specimen preparation

Experimental animals were sacrificed by anesthesia drug overdose at 1 day, 4 days, 10 days, 4 weeks, or 8 weeks after surgery (n = 1 dog per healing period).

Block sections that included segments of the grafts were dissected and fixed in 10% neutral buffered formalin for 10 days.

5. Radiographic evaluation

The fixed block specimens were scanned using a micro-CT system (SkyScan® 1072, SkyScan, Aartselaar, Belgium) at a resolution of 35 μm (100 kV and 100 μA). The scanned set of data were processed in DICOM format and the area of interest was reconstructed with OnDemand 3D® software (Cybermed, Seoul, Korea). The overall dimensional topography of the grafts and recipient beds was visualized with a 3D reconstructed image.

6. Radiographic analysis: micro-CT

The radiographic analysis was performed by one experienced researcher. The residual volume (in mm^3) of the block grafts was measured at the various healing periods. The radiographic measurements regarding the cross-sectional bone area (in mm^2) for each condition were performed in axial cross-sectional views. The residual bone height was measured along the fixation screw. The percentage of remaining bone height relative to the initial bone height was calculated in axial cross-sectional views as the distance from the bottom of the screw head to recipient bed (Fig. 3). The measurements were obtained from both sides of screw and the average was calculated.

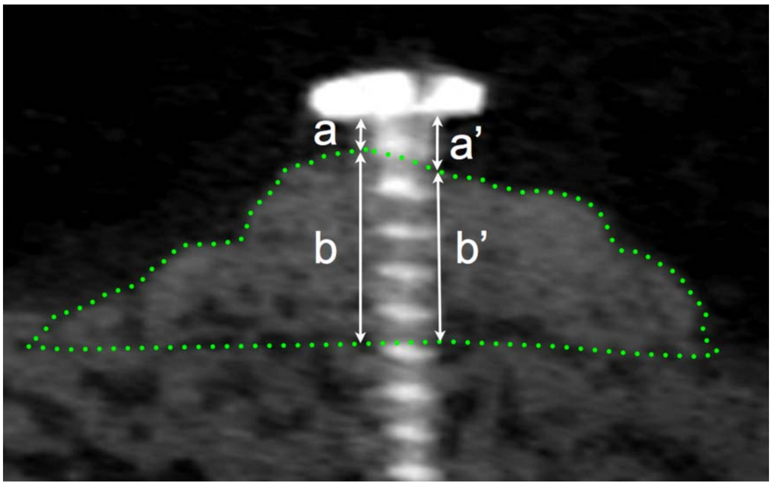


Fig. 3. Radiographic measurements. Cross-sectional bone area (mm^2): area surrounded with green dots. Residual bone height (%) = 100 X b/(a+b); (a+b) = distance from the bottom of the screw head to the recipient bed, b = distance from the recipient bed to the highest contact point of the residual graft and the screw. The opposite side of the screw was also measured (a', b').

III. Results

1. Clinical findings

Surgical wound healing was uneventful during the experimental period. No complications including wound dehiscence, severe swelling, and bleeding were observed.

2. Radiographic analysis: micro-CT

Early healing period: 1, 4, and 10 days

There was neither dimensional change nor integration between the graft and recipient bed in any of the four groups. A radiolucent space was observed between the graft and the recipient bed (Fig. 4 and 5).

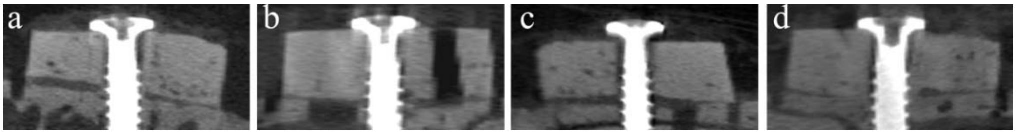
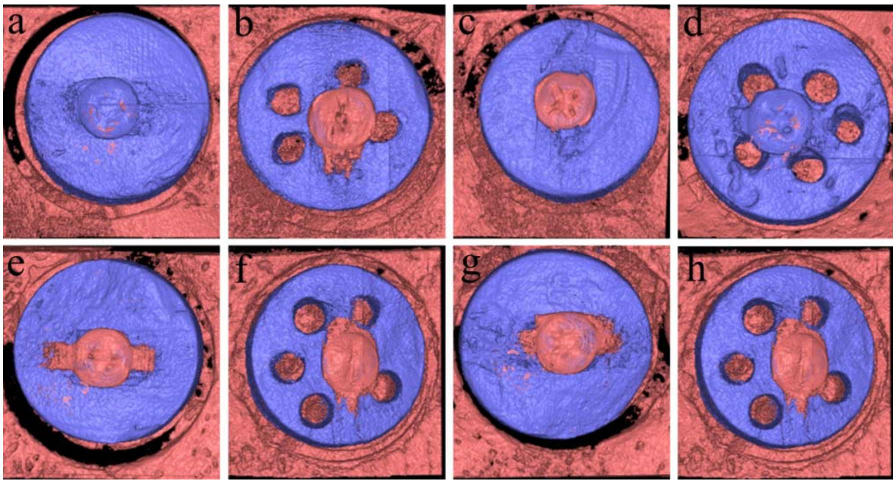


Fig. 4. Cross-sectional views of the four experimental conditions at 1 day of healing: (a) SGNPR, (b) PGPR, (c) SGPR, and (d) PGNPR. Radiolucent lines remain between the graft and the recipient bed.



Fig, 5. 3D reconstructed images of the four experimental conditions at 4 (a, b, c, d) and 10 days (e, f, g, h) of healing. (a, e) SGNPR, (b, f) PGPR, (c, g) SGPR, and (d, h) PGNPR.

Late healing period: 4 and 8 weeks

For all conditions, external resorption of the grafted block bone was first observed 4 weeks postoperatively. The bone resorption was the lowest at the central area in contact with the fixation screw, and the highest at the boundary area of the graft. Accordingly, the reabsorbed block bone appeared dome shaped in the cross-section (Fig. 6). Additional new bone formation was observed at the tenting area, with its flap lifted from the side of the block bone. Overgrowth of new bone beyond the screw head was observed in the PGPR and the PGNPR group (Fig. 6b, 6d, 6f, 6h).

At 8 weeks of healing, in the SGNPR treatment group the graft failed to integrate with the recipient bed (Fig. 7). The residual volume of block bone was higher (87.59 mm^3) in the PGPR condition with perforation of both the graft and recipient bed, and lower (42.02 mm^3) in the SGNPR condition, in which both the graft and recipient bed were intact (Table 1). The cross-sectional area and remaining height of the block bone were also highest (12.03 mm^2 and 70.37%, respectively) in the PGPR condition and the lowest (3.9 mm^2 and 23.52%) in the SGNPR condition (Table 2 and Fig. 8).

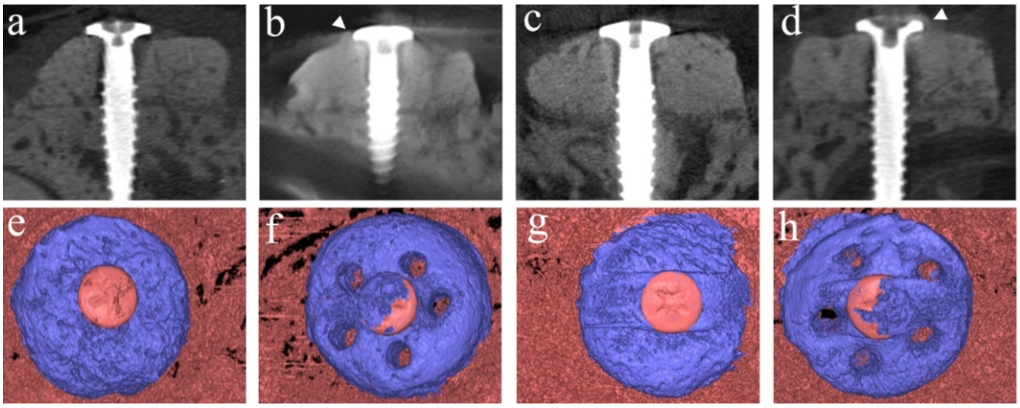


Fig. 6. Cross-sectional views (upper panels) and 3D reconstructed images (lower panels) made at 4 weeks of healing. (a, e) SGNPR, (b, f) PGPR, (c, g) SGPR, and (d, h) PGNPR. A dome-shaped resorption pattern was observed under all conditions. Arrow head: Notice the growth of new bone over the fixation screw in the perforated block graft groups (b, d, f, h).

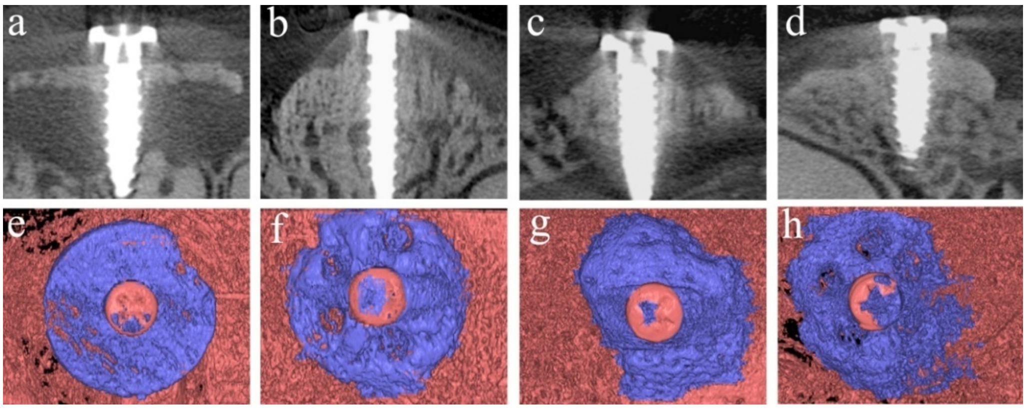


Fig. 7. Cross-sectional views (upper panels) and 3D reconstructed images (lower panels) at 8 weeks of healing. (a, e) SGNPR, (b, f) PGPR, (c, g) SGPR, and (d, h) PGNPR. The SGNPR condition exclusively failed to incorporate.

***Table 1* . Residual volume of block bone graft (mm^3)**

	SGNPR	PGPR	SGPR	PGNPR
1 day	130.57	124.17	125.08	118.39
4 days	127.6	100.05	118.32	127.34
10 days	129.94	90.56	123.33	114.93
4 weeks	97.42	102.89	121.47	122.87
8 weeks	42.02	87.59	65.63	77.21

SGNPR group: the solid block graft was fixed onto a nonperforated recipient bed; PGPR group: the perforated block graft was fixed onto a perforated recipient bed; SGPR group: the solid block graft was fixed onto a perforated recipient bed; PGNPR group: the perforated block graft was fixed onto a nonperforated recipient bed.

***Table 2* . Cross-sectional area in the five healing periods (mm^2)**

	SGNPR	PGPR	SGPR	PGNPR
1 day	17.66	18.20	14.94	16.33
4 days	16.64	13.06	17.84	19.87
10 days	16.56	16.18	16.91	19.25
4 weeks	11.27	16.20	14.69	15.32
8 weeks	3.90	12.03	6.82	5.04

SGNPR group: the solid block graft was fixed onto a nonperforated recipient bed; PGPR group: the perforated block graft was fixed onto a perforated recipient bed; SGPR group: the solid block graft was fixed onto a perforated recipient bed; PGNPR group: the perforated block graft was fixed onto a nonperforated recipient bed.

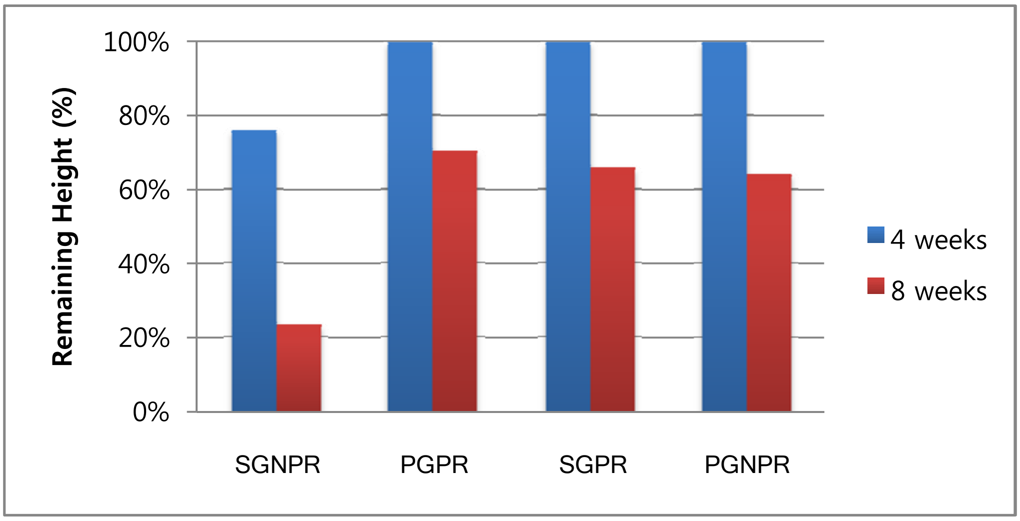


Fig. 8. Residual bone height (%) in cross-sectional view.

IV. Discussion

Autogenous bone grafts have been considered as the gold standard for alveolar ridge augmentation because of their osteogenic properties and excellent biocompatibility (Bauer & Muschler, 2000; Khan et al., 2005). However, whether cortical penetration is essential for the optimal graft condition remains controversial.

The process of perforating the cortical bone provides a passageway to help progenitor cells and blood vessels to easily reach the bone graft site. Moreover, as it creates a bleeding surface and blood clotting, growth factor and bone morphogenetic protein are induced (De Marco et al., 2005). Furthermore, cortical perforation may enlarge the surface area and, through the bone regenerated in the hole, it may strengthen the mechanical interlocking with the recipient site (Wang & Boyapati, 2006).

Conversely, many studies have found cortical perforation not to be significantly effective (Rompen et al., 1999; Lundgren et al., 2000; Adeyemo et al., 2008). It has been suggested that a rigid fixation and the intimate contact between the graft and recipient bed are more important than the cortical perforation of the recipient bed for bone graft incorporation (Adeyemo et al., 2008). However, most of those studies have focused on the presence of perforation of the recipient bed. In the free ABB, the blood vessels are severed and blocked from the donor site for a certain period. Moreover, a sufficient blood supply is not able to reach deep inside of the solid cortical bone in the early healing stage, since it originates from the recipient bone bed and covering flap to the outer surface of the free ABB.

It can be assumed that the central area of the grafted bone, which has little access to gases and nutrients, would become necrotic. It may explain the delayed healing and resorption. Therefore, the porosity of the grafted bone for the initial ingrowth of blood vessels into the deep region would be important in the maintenance of bone cell vitality.

In the present study, 3-mm-high cylinder-shaped grafts were obtained from the posterior mandible, which comprises intramembranous bone with a high cortical bone quality. Several authors have reported that cortical bone has better volume stability than cancellous bone, independent of its embryogenic origin (Ozaki & Buchman, 1998; Rosenthal & Buchman, 2003). Cancellous bone grafts could be revascularized more rapidly and extensively, but attendant osteoclastic resorption could also occur because of its inferior endurance to physiologic stress (Chen et al., 1994).

Various cortical perforation conditions in the recipient bed and the grafted ABB were alternatively prepared herein and the healing pattern was observed over time. Integration between the recipient bed and bone graft was observed at 4 weeks postoperatively in all groups. However, bone resorption was observed on the external surface of the block graft and gradual volumetric loss occurred, resulting in a dome-shaped appearance at 4 weeks of healing. Flap tensicon exerted on the external edge of the graft might have stimulated osteoclastic activity, resulting in more resorption peripherally than centrally, where the graft was supported by a fixation screw. Differences in bone volume maintenance were observed relative to the cortical perforation condition. At 8 weeks of healing, the residual bone volume (87.59 mm^3), cross-sectional area (12.03 mm^2), and residual bone height (70.37%) were larger in the PGPR condition than in the other conditions. At 4 weeks, additional new bone formation beyond the original boundary was observed in the upper part of the block bone, especially in the PGNPR and PGPR groups with perforation of the block graft.

In the present experiment, the PGPR condition exhibited the highest volume maintenance at 8 weeks of healing. This may be attributable to the perforation being performed on both the recipient bed and the block graft, which would enable a sufficient increase in the blood supply and, ultimately, early revascularization. The early revascularization of a block graft indicates early bone remodeling (Adeyemo et al., 2008). Therefore, woven bone could form more rapidly, and then its conversion into mature lamellar bone could lessen the volumetric loss. Furthermore, the revascularization of the graft can improve cell survival and support the healing of the block graft through "creeping substitution" where necrotic bone is substituted by viable new bone (McAllister & Haghighat, 2007). The early vascular penetration and nutrition of the graft are key factors for its integration into the recipient bed in preclinical study (De Marco et al., 2005). In the present study, the solid block graft that was fixed onto the nonperforated recipient bed was not integrated at 8 weeks of healing in the SGNPR condition. This is probably attributable to an insufficient vascular supply and resultant reduction in tissue metabolism.

In this study, only radiographic analysis was performed. Histologic evaluation will be prepared as a following further study and it will make up the deficiency of scientific merit. Also, the small sample size does not allow any statistical comparison. Further comparative studies with larger samples should be conducted so as to increase the statistical power.

Within the limitations of this study, it can be concluded that intentional cortical perforation on the recipient bed and block bone graft may influence volume maintenance of the graft.

V. References

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국문요약

개에서 블록형 자가골과 수혜부의 천공에 따른 영향. : 방사선적 분석

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목적: 이번 연구는 개에서 다양한 피질골 천공 조건하에서 이식된 블록형 자가골의 유착과 부피유지양상을 방사선적으로 평가하였다.

재료 및 방법 : 다섯마리의 잡견들이 사용되었다. 각 잡견에 네종류의 다르게 준비된 블록형 온레이 골이식을 시행하였다. : 건전한 골이식 블록을 (1) 피질골이 천공된 수혜부에 고정하거나(SGPR) 또는 (2) 천공되지않은 수혜부에 고정하였고(SGNPR), 천공된 골이식 블록을 (3) 피질골이 천공되지않은

수혜부에 고정하거나(PGNPR) 또는 피질골이 천공된 수혜부에 고정하였다(PGPR). 동물들은 술후 1 일, 4 일, 10 일,4 주, 8 주때 희생되었다. 표본들이 준비되었고, 컴퓨터 미세단층촬영술을 이용하여 방사선적 분석이 시행되었다. 블록형 이식골의 잔존골부피(RBV; mm^3), 횡단면 골면적(BA; mm^2), 잔존골 높이(RH; %)가 방사선적으로 측정되었다.

결과: 1 일, 4 일, 10 일의 치유기간에서는 수혜부와 이식골사이의 계면에서 골유착의 소견이 보이지 않았다. 그러나 4 주의 치유기간에서는 모든 그룹에서 골유착이 관찰되었다. 4 주의 치유기간때 까지 RBV, BA, RH 모두 점차적으로 감소되었다. 8 주때, PGPR 조건이 다른 조건에 비해 더 높은 RBV, BA, RHF 를 보였고, 반면에 SGNPR 조건은 가장 낮은 RBV, BA, RH 를 보였다.

결론: 이번 연구의 범위내에서는, 수혜부와 블록형 자가골에 의도적으로 피질골 천공을 시행하였을때, 이식골의 부피가 보다 더 유지되도록 영향줄 수 있다고 결론지을 수 있다.

핵심단어 : 치조능 증대, 골재생, 피질골천공, 개, 방사선 미세단층촬영