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The effect of particulate dentin-plaster of Paris combination with/without fibrin glue in the treatment of bone defects around implants

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임플란트 주위 골결손부 치료시 치아 회분말 및 연석고, 피브린 글루의 효과

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이 논문을 치의학 박사학위신청 논문으로 제출함.

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최 동 국

최동국의 박사학위논문을 인준함

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본 연구의 목적은 성견에서 임플란트 골결손부 치료시 치아 회분말 및 연석고, 피브린 글루의 효과를 알아보는 데 있다.

4마리의 잡종 성견이 암수 구별없이 선택되었고, 실험부위로 좌우측 대퇴부가 이용되었다. 성견의 대퇴부에 골 결손부를 형성하기 위하여 4mm의 수직 결손부(3개의 thread의 노출)와 6mm의 수평 결손부를 원형으로 trephine bur를 이용하여 형성하였다. 총 24개의 직경 4mm, 길이 10mm의 Self tapping type Neoplant 임플란트를 각각의 대퇴부에 3개씩 식립하였다. 각각의 성견은 아래와 같은 3가지 방법으로 처치하였다; (1) 아무런 처치를 하지 않은 것, (2) 치아-연석고 회분말을 이용하여 이식, (3) 치아-연석고 회분말과 Fibrin Sealant(Tisseel® Baxter AG, Vienna, Austria)을 이식한 것. 모든 임플란트는 submerge되었고, 연조직은 긴장 없이 봉합하였다. 실험후 조직형태학적 검사를 위한 표본채취를 위하여 4주후 2마리, 8주후 2마리씩 희생하여, 조직학적 검사 및 조직형태학적 검사를 시행하였다.

실험 2군과 실험 3군은 대조군에 비하여 신생골 충전율이 증가하고 신생골 충전 높이(level)가 높아지는 경향을 보였다. 그리고, 각군은 시간의 경과에 따라 더 좋은 신생골 충전율과 충전 높이(level)가 높아지는 경향을 보였다.

치아 회분말 및 연석고 매식을 피브린 글루와 병용시 더 좋은 결과를 기대할 수 있을 것으로 사료된다.

Introduction

Implant placement requires adequate bone volume. When the bone volume is insufficient in height and width, autogenous bone grafting or bone substitutes is essential. Several techniques have been advocated for the generation of new bone tissue within or around a tentative implant site. Such techniques have utilized graft material alone or in combintation with barrier membranes, platelet rich plasma, and so on[1].

Autogenous bone graft is still considered as the "gold standard" for bone defect augmentation, owing to its osteogenic, osteoinductive, and osteoconductive properties. However, autogenous bone grafting has some disadvantages. It sometimes necessitates extra-oral donor sites and the quantity of bone harvested from an intra-oral site may be insufficient. Moreover, the morbidity risk should be considered for both intra- and extra-oral sites[1].

Alloplastic grafts include dense and porous hydroxyapatites, tricalcium phosphates, a mixture of both materials, and a derivative of natural coral. These materials present an advantage that involves the absence of an associated immunological reaction or risk of disease transmission, as well as their biocompatibility and lack of toxic effects. They promote bone regeneration when they are grafted in a healthy site. Hydroxyapatite, alone or in association with other materials, has been used in bone grafting[1].

Fibrin sealants of various formulations have been used widely in surgical procedures throughout the body as a means of establishing hemostasis and for tissue approximation. Fibrin sealants have been used at such diverse locations as the heart and pericardial cavity, lung, bowel anastomoses, ovary, nasal sinuses, and skin. Formulations include both autologous preparations, which require peri-operative preparation, and prepackaged commercial kit[2].

The osteogenic capability of particulate dentin-plaster of Paris with and without fibrin glue to fill the bone defects around titanium dental implants has not been previously reported.

The purpose of this study was to evaluate the effect of particulate dentin-plaster of Paris with and without fibrin glue on bone healing and new bone formation around titanium dental implants in a canine model.

Materials and Methods

Materials studied

This study was approved by the Animal Research Committee of Chosun University. Four healthy, mature (one year old) male and female mongrel dogs were selected. Preoperatively, the dogs were anesthetized with an intramuscular injection of ketamine HCl (Ketalar®, Yuhan, 10 mg/kg) and xylazine (Rompun®, Bayer, 3 mg/kg). During anesthesia induction, each dog received cefazoline (22 mg/kg, IV). To aid hemostasis in the areas of the planned soft tissue incisions and dissection, 2ml 2% lidocaine HCl with 1:100,000 epinephrine was injected in the ilium.

The surgical procedure was initiated by an incision, and mucoperiosteal flaps were gently raised. The three defects were created in each animal. Three circular bone defects measuring 4 mm apicocoronally, and 6 mm mesiodistally and buccolingually were surgically prepared in iliac crest sites in each animal.

A total of 24 Neoplant dental implants (Neobiotech Co., Seoul, Korea) were used as the experimental implants. The implants were self-tapping screw-type implants, 10 mm in length and 4 mm in diameter, all made of commercially pure titanium. Each titanium implant was then placed centrally in the defect in such a way that 3 threads were exposed and the cover screws were at the level of the intact proximal part of the crest. In each dog, the defects were treated with one of the following three treatment modalities: (1) no treatment (control); (2) grafting with particulate dentin-plaster of Paris; (3) grafting with particulate dentin-plaster of Paris and fibrin glue.

All of the implants were submerged with tension-free mucoperiosteal flaps using the vertical mattress suturing technique.

Particulate dentin preparation procedure

Particulate dentin was produced using the following method: first, removed the foreign matter and soft tissue attached to the teeth's surface by soaking the relatively good teeth in hydrogen peroxide. Next, the teeth were ground using a mortar and pestle after heating them in a furnace at a high temperature (2192°F) for two hours. They were ground as completely as possible. The teeth were then filtered using a mesh tray (Sieve No. 100), and the filtered powder was ground minutely two or three times. The final particle size of the particulate dentin was 0.149 mm. Disinfection was

carried out in an autoclave after placing the powder into a beaker filled with distilled water. After disinfection, the tap water and floaters were carefully removed using a pipette, and the distilled water and residue were placed back into the beaker which was shaken. Sterilization was done after storing the solution for one day. This autoclaving process was repeated five times to completely remove any foreign matter. After the final sterilization, the distilled water and residue were removed and dried using a drying oven. After autoclaving, the yielded powder was used as implant material. The prepared materials were kept using ethylene oxide after disinfecting them. Measurements of their weight were made for convenient use.

Particulate dentin-plaster of Paris preparation procedure

The particulate dentin and plaster of Paris were mixed using saline at a ratio of 2:1 by weight and placed into the defect. Once it had dried, the material was sculpted with a bur so as to match the contour of the remaining bone.

Fibrin glue

The fibrin sealant, Tisseel Duo Quick (Baxter AG, Vienna, Austria), was used. It consisted of deep-frozen Tisseel and thrombin solutions in two disposable syringes. The Tisseel solution contained 100-130 mg total protein, 75-115 mg clottable protein, 70-110 mg fibrinogen, 2-9 mg fibrinonectin, 10-50 IU factor XIII, 40-120 μg plasminogen, 3000 KIU bovine aprotinin, 10-20 mg human albumin, 15-35 mg glycine, 2-4 mg NaCl, 4-8 mg sodium citrate, 0.2-0.4 mg Polysorbate 80, 15 mg creatin monohydrate, and water for a total injection volume of 1 ml. The thrombin solution contained 500 IU thrombin, 50 mg human plasma protein, 5.88 mg CaCl2, 10 mg NaCl, 3 mg glycine, and water for a total injection volume of 1 ml. The Tisseel and thrombin solutions were mixed to form a fibrin clot. The approximate time for resorption was about 2 weeks.

Histologic procedure

At 4 and 8 weeks after implantation, the animals were sacrificed by perfusion with formalin fixative through the left ventricle of the heart. A total of 24 implants were retrieved. The implants and surrounding tissues were immediately washed in saline solution, and then immediately fixed in 70 % alcohol at 4℃ for six days. The specimens were dehydrated in an ascending series of alcohol rinses and embedded using a process which produces thin

ground sections with the glycol-methacrylate resin (Spurr Low-viscosity Embedding Media, Polysciences, Warrington, PA). After polymerization, the specimens were sectioned along their longitudinal axis with a high-precision diamond disc (Low speed diamond wheel saw 650, SBT, San Clemente, CA) at approximately 200 ㎛, and ground down to approximately 30 ㎛ using a lapping and polishing machine (OMNILAP 2000, SBT, San Clemente, CA).

Histomorphometry

Three slides were created for each implant. The slides were stained with bone stain (Villanueva osteochrome bone stain, SBT, San Clemente, CA) according to the manufacturer's instructions. The slides were observed in normal transmitted light under an Olympus BX50 (Olympus, Tokyo, Japan). The histomorphometry was performed with a Microvid system (Leitz, Wetzlar, Germany) connected to an IBM personal computer.

● Rate of new bone formation = newly formed bone/area outside the implant thread X 100 (%)

● NB: new bone
● NB filling (%): The rate of new bone formation in defect area
● Filling level: The value measured the level of new bone filling rate in the defect area independent from NB filling (%).

[Examples of Filling level]
level 1: cases covered the cover screw
level 2: top of the cover screw
level 3: bottom of the cover screw
level 4: bottom of the head
level 5: neck
level 6: thread 1
level 7: thread 2
level 8: thread 3

Statistics

The data were analyzed using the Kruskal-Wallis test in the Statistical Package for the Social Sciences (SPSS) for Windows, version 7.5 (SPSS, Korea). Analysis of variance (ANOVA) with a multiple comparison test was used

for inter-group comparison among the groups. The time periods in each group were compared with the Wilcoxon rank test. Values of $p < 0.05$ were considered statistically significant.

Results

Group 1. At 4 weeks, the level of new bone filling in the defect area was meager, and the level of new bone formation was very low in some cases, on the other hand, cases with unexpectedly high rate of filling level and the formation level were observed. In addition, comparing the filling rate and the formation level by dividing them to the right and the left, the difference was large, and thus cases showing the noticeably good filling rate and the formation level in one side in comparison with the contralateral side were observed (Fig. 1). At 8 weeks, the new bone filling rate and the formation level in the defect area were very high, and in comparison with the 4 weeks group, the difference of the lingual side and the contralateral side was markedly reduced (Fig. 2).

Group 2. At 4 weeks, in comparison with the group 1, the formation level were increased, and the difference of the right and the left side was decreased (Fig. 3). At 8 weeks, in comparison with the 4 weeks group, the bone filling rate and the bone formation level were improved, and the difference of the lingual side and the contralateral side was decreased (Fig. 4).

Group 3. At 4 weeks, in comparison with the group 1, the new bone filling rate and the formation level were improved, and the difference of the lingual side and the contralateral side was reduced, nonetheless, in comparison with the group 2, a significant difference was not shown (Fig. 5). At 8 weeks, the new bone filling rate and the formation level were detected to be increased, nevertheless, in comparison with the 4 weeks group, a marked improvement of the new bone filling rate and the formation rate was not shown (Fig. 6).

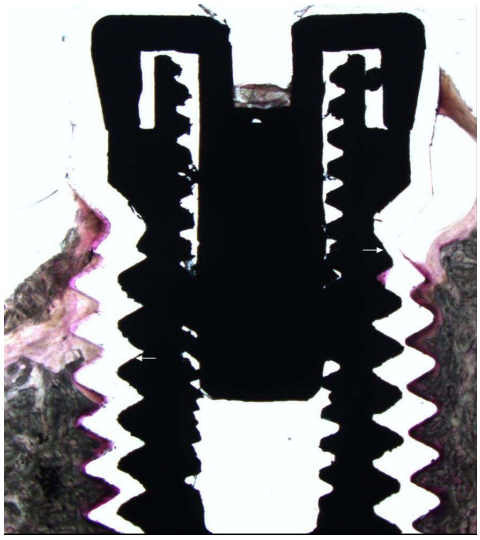

Fig. 1. Control group at 4 week. The levels of a new bone filling were 1st (level 6) and 3rd (level 8) thread of the right and left side, respectively (Villaneuva bone stain, Original magnification ×12.5).

Fig. 2. Control group at 8 week. The levels of a new bone filling were the neck (level 5) and mid-portion of the head (level 3-4) of the right and left side, respectively. Arrows indicate the levels (Villaneuva bone stain, Original magnification ×12.5).

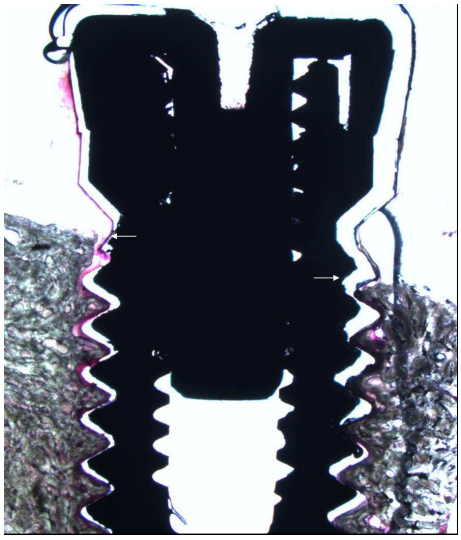

Fig. 3. Group 2 at 4 week. The levels of a new bone filling were the neck (level 5) and lower portion of the 1st thread (level 6) of the right and left side, respectively.
Arrows indicate the levels (Villaneuva bone stain, Original magnification ×12.5).

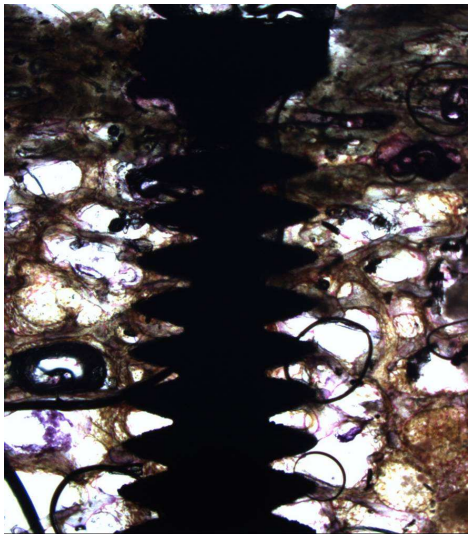

Fig. 4. Group 2 at 8 week. Newly formed bone covered the cover screw (Villaneuva bone stain, Original magnification ×12.5).

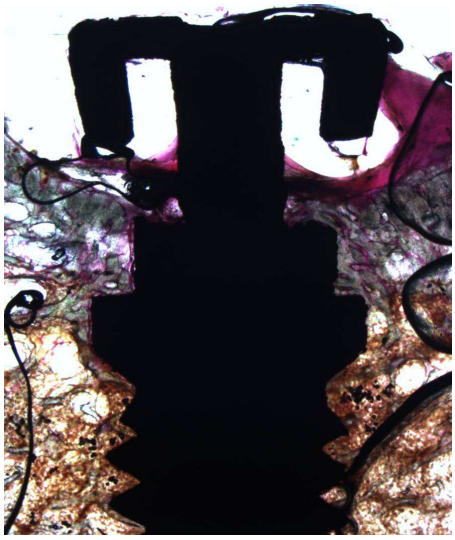

Fig. 5. Group 3 at 4 week. The levels of a new bone filling were the cover screw (level 2-3), respectively. Arrows indicate the levels (Villaneuva bone stain, Original magnification ×12.5).

Fig. 6. Group 3 at 8 week. The level of a new bone filling is the cover screw (level 2-3) of the right side. However, about 65% of the cover screw from the left side is covered (asterisk) by new bone (level 1). Arrow indicates the level (Villaneuva bone stain, Original magnification ×12.5).

In the experiment groups (groups 2 and 3), in comparison with the control group, a trend that the new bone filling rate was increased and the new bone formation level was increased was shown. In addition, with time, a trend of a better new bone filling rate and the increased formation level was shown (Table 1).

Table 1. Mean percentages of new bone formation in Groups 1, 2, and 3, at 4 and 8 weeks after placement

	Mean ± SD		
Time period	Group 1 (Control)	Group 2	Group 3
4 weeks	37.3 ± 9.4	44.5 ± 11.4*	54.0 ± 13.4*,+
8 weeks	38.6 ± 6.7	45.5 ± 12.0*	58.0 ± 11.6*

*Statistically significant difference relative to Group 1.

+Statistically significant difference relative to Group 2.

SD = standard deviation.

Discussion

The threaded portion of all Neoplant dental implants in the present study were osseointegrated. The results demonstrated that surgically created bone defects, similar to those observed around failed dental implants, healed with complete bone fill and osseointegration to the implant surface when grafted with a particulate dentin-plaster of Paris, whether or not the defect was grafted with fibrin glue.

Tooth ash (particulate dentin, tooth particles) is derived from teeth and composed mainly of HA. Particulate dentin with a uniform particle size is prepared from extracted teeth by washing, ashifying at high temperatures between 900 to 1300℃ for 90 to 120 minutes, grinding, and removing any impurities[3].

Plaster of Paris is readily available, easily sterilized, inexpensive, completely and rapidly resorbable, and biocompatible, and has been shown to be well tolerated by tissues. The combined implant material using tooth ash and plaster of Paris offers the following benefits. (1) safe for patients (no significant foreign-body reaction or infection), (2) high osteoconductive capacity, (3) absorbable property, (4) easy to handle, (5) prevention of environmental pollution by retreating the waste material, and (6) the cost effectiveness[2-5].

Najjar et al.[6] attempted to determine whether the addition of calcium sulfate to HA implant material would improve its working properties without adversely affecting its osseointegration capability. No sign of extensive chronic inflammatory response was detected. The highest rate of bone ingrowth occurred with an HA composite (HA plus calcium sulfate), followed by HA alone. Bone was deposited directly on the surface of HA and HA composite implant materials. Calhoun et al.[7] noted that natural gypsum showed hardly any tissue reactions, such as inflammation. McKee and Bailey[8] observed that the worst problem resulting from the use of Plaster of Paris was infection. They also reported that because such infection could be controlled effectively, successful replacement of the calcium sulfate for new bone formation could occur either with or without the presence of the periosteum.

Kim et al.[9] microscopically examined toothash and plaster of Paris. Implanted particles were divided into small particles and the number of particles decreased gradually over the first eight postoperative weeks. Most

of the implanted sites were repaired by newly formed bone by the 8th postoperative week.

Recently, fibrin sealants that typically contain high physiological concentrations of fibrinogen and thrombin have been investigated as matrices to facilitate the delivery of cells within biodegradable scaffolds for tissue engineering applications[10].

Different biomaterials such as coral, bone-derived materials, bioactive glass ceramics, and synthetic calcium phosphate have been mixed with fibrin sealant, resulting in a combination of the biological properties of the two components. This type of association has not produced identical results in all studies. In the past for some, the addition of fibrin sealant to the biomaterial failed to produce any significant, positive effect on osteointegration, whereas others found a positive impact on bone colonization. Despite the negative biological effects reported previously, bioceramic-fibrin composites have been widely used in various types of bone surgery because they are easy to manipulate. In particular, the intra-operative preparation of these composites makes it possible to add bone growth factors or autologous osteoprogenitor cells prior to bone reconstruction. The bone growth factors and autologous osteoprogenitor cells associated with the bioceramic-fibrin composites should provide surgeons with tissue engineered grafts with enhanced osteointegrative properties[11].

Fibrin tissue adhesive material (FAM) is available as fast (about 5 seconds) and slow (about 1 min) setting types. FAM is supplied in two vials. One contains fibronectin, fibrinogen, factor XIII, and aprotinin (an anti-fibrinolytic agent), while the other contains thrombin and calcium chloride. The components become a gel when mixed. FAM acts as both a tissue adhesive and a hemostatic material. It also enhances wound healing[12,13].

In addition to the physical benefit of using fibrin glue in reconstructive bone surgery, the glue also accelerates the bone graft healing process[14,15], as the fibrin network acts as a scaffold for invading cells[16] and as a carrier for bone induction[17].

FAM might act as a material for creating space. It might also act in conjunction with a growth factor, such as bone morphogenic protein (BMP), insulin-like growth factor, or platelet-derived growth factor.

In the present study, nongrafted defects demonstrated bone regeneration

in their lower portions only. All of the bone defects grafted with particulate dentin-plaster of Paris material, showed complete healing with bone-fill. Osseointegration between the bone and implant was frequently established. The majority of particulate dentin-plaster of Paris particles were incorporated into the newly formed bone, with an intimate contact with the implants. In addition, there was a progressive increase in the new bone volume over time.

Conclusion

The histomorphometric results showed significantly higher percentages of bone-implant contact. This study confirmed that the portion of the experimental implants placed in the host bone, osseointegrated, and a large amount of bone contact was achieved with the implant. Results of the present study suggest the efficacy of particulate dentin-plaster of Paris in the treatment of bone defects around dental implants. Because of the high rate of new bone formation with no major observed side effects, it was concluded that particulate dentin-plaster of Paris with fibrin glue has the potential to become a novel treatment method for bone defects around dental implants.

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저작물 이용 허락서

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본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

2007년 4월 일

저작자: 최 동 국 (서명 또는 인)

조선대학교 총장 귀하