日本財団 助成事業
The Nippon Foundation

２００３年度日中医学協会共同研究等助成事業報告書

－在留中国人研究者研究助成－

２００４年 ３ 月 ６ 日

財団法人 日中医学協会
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１．研 究 テ ー マ

口腔内インプラント材料の生体内安定性に関する実験的研究

２．本年度の研究業績

(1) 学会・研究会等における発表 (有)・無 （学会名・演題）

12th EAO Annual Scientific Congress (October 9-11, 2003, Vienna, Austria)
Grafting of deproteinized bone particles inhibits bone resorption after maxillary sinus floor elevation.

(2) 学会誌等に発表した論文 (有)・無 （雑誌名・論文名）

Journal of Oral Pathology & Medicine, 32 (8): 499-501, 2003.
Pleomorphic adenoma of the submandibular salivary glands with marked ossification.

Clinical Oral Implants Research, 15 (1): 126-133, 2004.
Grafting of deproteinized bone particles inhibits bone resorption after maxillary sinus floor elevation.

3．今後の研究計画

インプラントの固定には、骨の調和を達成することが基本的要件の一つである。低密度の骨においては適切な固定を達成するのが難しいことが多い。これまでの長期経過の実験的研究で、再生骨は吸収され、補填材周囲組織に脂肪組織が多くなり、緻密骨はほとんど消失した、その原因に再生骨への栄養血管の育成の不十分さが考えられた。今後の研究目的として、再生骨の臨床的な長期安定性の保持に注目したい。VEGF はその増殖作用が血管内皮細胞に特異的であり、遺伝子治療を考えていくうえで理想的な因子であると考えられている。より早期に安定した骨形成と長期の骨の維持を目指すために、血管新生療法として、血管内皮増殖因子(VEGF)を用いて実験を行う。

4．指導責任者の意見

徐輝君は、大学院では、文字通り休日返上で昼夜を問わず研究に取り組んで、同僚の日本人の院生以上の成果を納めている。2003 年度の貴協会の助成金を受け、研究に集中することを可能にし、平成１５年度の「東北大学総長賞」を授与されることになった。現在投稿中の論文には、日中医学協会助成金によるところが多く、感謝しています。徐輝君の研究成果は、国際的にも認められ、2003 年には、インプラントでは世界で一番権威のあるヨーロッパ 12th EAO Annual Scientific Congress で発表し、徐輝君は既に研究者としての能力を身に付け、今後が期待される逸材です。

指導責任者氏名 大家 淸 印

5．研 究 報 告 書

別紙「研究報告書の作成について」に倣い、指定の用紙で作成して下さい。

研究発表または研究状況を記録した写真を添付して下さい。

※研究成果を発表する場合は、発表原稿・抄録集等も添付して下さい。

※発表に当っては、*日中医学協会助成金による*旨を明記して下さい。

—日中医学協会助成事業—

口腔内インプラント材料の生体内安定性に関する実験的研究

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Abstract

This experimental study evaluated the long-term outcome of deproteinized bone particles in a rabbit model for maxillary sinus augmentation. Histologically, 8 weeks after implantation, large amounts of newly formed bone showed many interconnections and appeared in most parts of the implant cavity. Sixty-four weeks after implantation, most of the newly formed bone in the augmented spaces had been resorbed. Only a few thin, and slender regions of newly formed bone were embedded in fatty tissue. Histomorphometric analysis revealed a significant decrease in bone area and a significant increase in bone marrow space area with time. There was no significant change in the deproteinized bone particle area. We conclude that deproteinized bone particles do not resorb with time and that the newly formed bone in the augmented space after implantation is not stable on a long-term basis in rabbits undergoing maxillary sinus augmentation.

Key Words sinus augmentation; deproteinized bone; particle; rabbit

Introduction

Sinus augmentation is an established method used to enhance vertical bone height in the posterior region of the maxilla, thereby increasing primary stability before implant insertion. Various materials have been used for bone grafts in sinus lift operations designed to increase vertical bone height and promote osteogenesis.

Deproteinized bone, one type of xenograft, has been shown to be a safe and biocompatible bone graft material with osteoconductive properties. It is frequently used for maxillary sinus lift procedures because of its natural morphologic characteristics, complete deproteinization of inorganic components, and lack of antigenicity. Several animal studies have shown this material to be promising as compared with other bone graft materials. However, the long-term outcome of deproteinized bone particles in the augmented maxillary sinus is not well understood.

We therefore histologically and histomorphometrically evaluated the long-term outcome of deproteinized bone particles in an experimental model of maxillary sinus augmentation.

Material and methods

Surgical procedures

Twenty adult male Japanese white rabbits (average weight, 3.0 kg) were used. The rabbits were anesthetized with pentobarbital sodium (0.5 mg/kg) intravenously, and 0.5 ml of 1% lidocaine with epinephrine (1:100,000) was injected subcutaneously in each surgical field for local anesthesia. The nasal bone and nasoincisal suture line were exposed via a perpendicular incision. With the use of a round bur, a nasal bone window was outlined and a fenestra was made by osteotomy during

continuous cooling with sterile saline solution. Care was taken during this procedure to avoid damaging the antral mucosa. Once the outline was completed, a Freer elevator was used to gently push the antral mucosa inward. The mucosa was then elevated from the floor, lateral walls, and medial wall of the antrum to provide a large compartment for graft placement. The space was filled with the deproteinized bone particles. The particles were gently packed into the cavity without compression. The defect was covered with a membrane (Bio-Gide, Geistlich, Wolhusen, Switzerland) to prevent fibrous connective tissue ingrowth into the augmented space.

Tissue preparation

The rabbits were anesthetized intravenously with pentobarbital sodium and were killed 4, 8, 16, 32, and 64 weeks after operation. For histological examination, the rabbits were exsanguinated and perfused via the jugular veins with 2% paraformaldehyde solution in 0.1 M cacodylate buffer (pH 7.4). The implant sites were dissected free, fixed in the same solution for 48 hours at 4℃, and decalcified with 10% ethylenediaminetetraacetic acid (EDTA) at 4℃. The specimens were then dehydrated in a graded series of ethanol, embedded in paraffin, and sliced into sections about 3 μ m thick. The sections were stained with hematoxylin-eosin and examined by light microscopy.

Histomorphometric analysis

Each image was copied on color reversal film, digitized as a 256×256 array of 8-bit density values, and transferred to a microcomputer for histomorphometric analysis. The Cadkey Image (Cadkey System Corp., Tokyo, Japan) program was used for analysis. The following histomorphometric measurements were made: bone area (percentage of newly formed bone area to total measured area); bone marrow space area (percentage of bone marrow space area to total measured area); augmented height (maximal height of the augmented space); and particle area (percentage of particle area to total measured area).

Statistical analysis

The statistical significance of differences between implantation times was analyzed by analysis of variance (ANOVA) with Tukey's method. The level of statistical significance was defined as $p < 0.05$. All the data are expressed as means ± standard deviation.

Results

Histological findings

4 weeks after implantation

Thick newly formed bone was observed adjacent to the cortical bone wall of the space. There was a tight interface between the newly formed bone and particles, without any gaps. In the center of the cavities, the particles were surrounded by fibrous connective tissue.

8, 16 weeks after implantation

Eight weeks after implantation, large amounts of newly formed bone showed many interconnections and appeared in most parts of the cavities. New formed bone was present between and around the deproteinized bone particles. Bone marrow was now developing in augmented space and sporadically communicated with the cortical bone wall of the cavity. Sixteen weeks after implantation, a larger bone marrow space was found in the augmented space.

32, 64 weeks after implantation

Thirty-two weeks after implantation, the thickness of the newly formed bone had decreased, and large bone marrow spaces were frequently seen. Sixty-four weeks after implantation, most of the newly formed bone in the augmented spaces had been resorbed. Only a few thin, slender regions of newly formed bone were embedded in fatty tissue. Bone marrow space containing many fat cells

was present in most parts of the cavities.

Histomorphometrical analysis

Bone area

Bone area significantly increased from 4 to 8 weeks, and then significantly decreased from 16 to 64 weeks. There was no significant difference between 8 and 16 weeks. Significant differences were observed between 4 and 8 weeks ($p < 0.01$), 4 and 16 weeks ($p < 0.01$), 4 and 64 weeks ($p < 0.001$), 8 and 32 weeks ($p < 0.001$), 8 and 64 weeks ($p < 0.001$), 16 and 32 weeks ($p < 0.001$), 16 and 64 weeks ($p < 0.001$), and 32 and 64 weeks ($p < 0.01$) after implantation.

Bone marrow space area

Bone marrow space area significantly increased with time. Significant differences were observed between 4 and 16 weeks ($p < 0.01$), 4 and 32 weeks ($p < 0.001$), 4 and 64 weeks ($p < 0.001$), 8 and 32 weeks ($p < 0.001$), 8 and 64 weeks ($p < 0.001$), 16 and 32 weeks ($p < 0.001$), 16 and 64 weeks ($p < 0.001$), and 32 and 64 weeks ($p < 0.001$) after implantation.

Augmented height and particle area

Augmented height and particle area did not change significantly with time.

Discussion

Deproteinized bone, a pure anorganic bone graft material, is safe and has been shown to have many advantages over other materials in experimental models. This material is thought to be resorbed with time. In our study, however, the deproteinized bone particles were apparently unaffected by remodeling. There was no significant change in particle area from 4 to 64 weeks. This finding suggests that deproteinized bone particles are not resorbed with time.

Biomechanical studies have found that the stability of implants is related to the mechanical properties of the surrounding bone. For instance, implant stability is better in lamellar bone than in cancellous bone. Therefore, an understanding of the different quantities and qualities of newly formed bone is important. Another important determinant of long-term outcome in patients who undergo sinus augmentation is maintenance of augmented height. This is particularly important if an implant is lost or further implant placement is considered in the future. In our study, there was an apparent early increase and subsequent decrease in bone area, but the augmented height was unchanged with time. The decrease in bone area was accompanied by an increase in marrow adipose tissue.

Bone loss associated with conditions such as osteoporosis, age-related osteopenia, or immobilization may be accompanied by an increase in marrow adipose tissue. The role of adipocytes in marrow is not yet understood, but an excess of marrow adipose tissue is considered to negatively affect the long-term mechanical strength of the skeleton. It is often difficult to obtain implant anchorage, in bone that is not very dense.

Biochemical studies have suggested a relation between mechanical stress and bone metabolism. Tissue is likely to mature after functional loading. However, information on the optimal time for implantation is scant. On the basis of bone remodeling, 8 weeks after grafting is regarded to be an appropriate time for implant placement in this experimental model.

Conclusion

Deproteinized bone particles are not resorbed with time. Newly formed bone in the augmented space after the deproteinized bone implantation is not stable on a long-term basis in rabbits

undergoing maxillary sinus floor elevation.

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作成日：2004年2月19日

Grafting of deproteinized bone particles inhibits bone resorption after maxillary sinus floor elevation

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Introduction

Maxillary sinus floor augmentation is an established method for harvesting sufficient vertical bone from the posterior region of the maxilla to achieve good primary stability before implant insertion. Various graft materials, including autografts, allografts, alloplasts, and xenografts, have been used for sinus augmentation to increase bone volume and height. However, some materials, such as freeze-dried demineralized bone and autogenous bone, cannot withstand sinus pressure during the first several weeks and lose density and height over time. In recent years, sinus augmentation using deproteinized bone has been successful both in animal-based research work with monkeys, dogs, sheep and rabbits and in therapeutic applications in humans. The present study was designed to examine the value of deproteinized bone particles on bone resorption in rabbits undergoing maxillary sinus grafting.

Material and methods

Experimental animal

Adult male Japanese white rabbits (average weight: 3.0 kg)

Preparation of bone graft materials

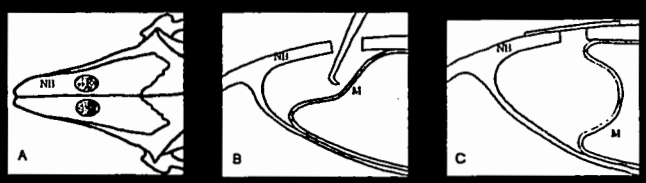
The deproteinized bone was prepared according to the method described by Matsuda et al.

1. Removal of protein (1% NaOH, H$_2$O$_2$)
2. 600°C (3.5hrs) → 1,100°C (3.5hrs)
3. Particle size: 300-500 μm

Histomorphometric analysis

Augmented height (the maximal height of the augmented space)
Bone area (percentage of newly formed bone area to total measured area)
Osteoclast number (average number of osteoclasts per millimeter of bone)
Particle area (percentage of particle area to total measured area)

Surgical Procedures

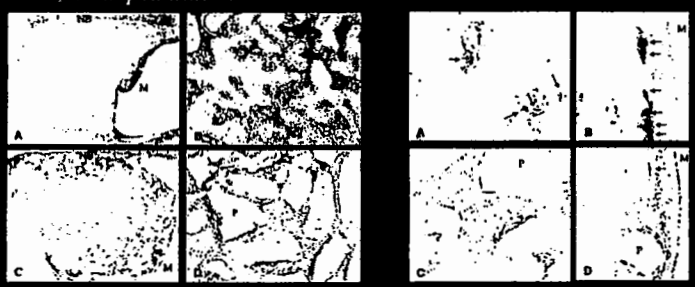


Schematic drawing of the surgical procedures. (A) The demineralized bone on the dorsal surface of the fibrous shell. (B) The elevated sinus membrane. (C) The sinus cavity filled with graft materials. NB: nasal bone; P: osteotomy; M: membrane.

Results

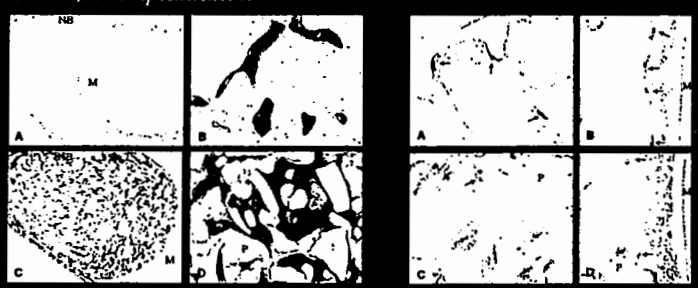
Histological findings

2w after implantation



hematoxylin-eosin: A & C: ×25, B & D: ×40 TRAP staining: A-D × 20

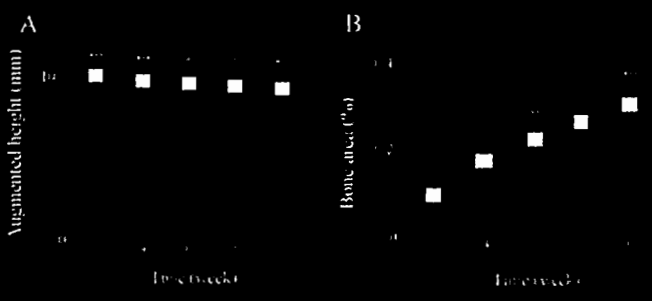
10w after implantation



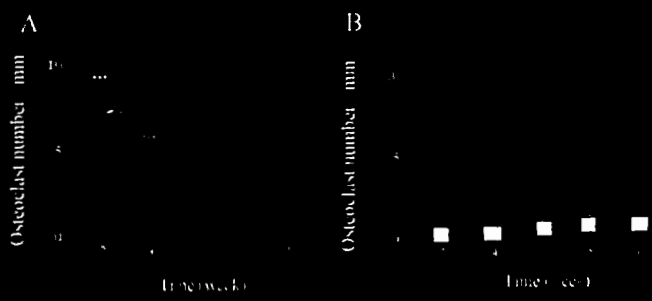
hematoxylin-eosin: A & C: ×25, B & D: ×40 TRAP staining: A-D × 20

A & B: Blood clots group; C & D: deproteinized bone particle group; NB: nasal bone; M: elevated sinus membrane; P: particle; Arrows: positive TRAP stained osteoclasts

Histomorphometric analysis



Histomorphometric measurement of augmented height (A) and bone area (B): blood clots group; ■: deproteinized bone particle group; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; n = 10



Histomorphometric measurement of osteoclast number: (A) blood clots group; (B) deproteinized bone particle group; ▲ & ■: in the augmented space; △ & □: adjacent to the elevated sinus membrane side; ***: $p < 0.001$; n = 10

Histomorphometric measurement of particle area; n = 10

Conclusion

Our results demonstrate that slowly resorbed deproteinized bone particles promote the stable augmentation of the maxillary sinus floor and inhibit the resorption of the newly formed bone.