Bone Engineering Using Human Demineralized Dentin Matrix and Recombinant Human BMP-2

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Abstract : We first confirmed the osteoinductive property of human demineralized dentin matrix (DDM) histologically. Human DDM was prepared from vital, extracted teeth and implanted into the subcutaneous tissue in nude mice. The shape of the DDM was a particle type and its size varied from 0.4 to 0.8 mm. The hard tissue induction by 70 mg of the DDM was estimated at 4 weeks after implantation. The DDM induced bone and cartilage independently. In addition, the time-course of the bioassay by recombinant human BMP-2 (5.0μγ)/ DDM (70mg) was estimated in rat subcutaneous tissues. Histological examination showed that the BMP-2/ DDM induced bone and cartilage and the DDM were gradually replaced by new bone. The morphometric analysis demonstrated that the BMP-2/DDM showed 79.0% in the volume of bone and marrow, and 21.0% in that of DDM at 32 weeks. These results indicate that human DDM particles are osteoinductive matrics and the DDM might be effective as a carrier of BMP-2 for bone engineering.

Keywords: Dentin; Human demineralized dentin; BMP-2; Bone induction

Introduction

The history of bone morphogenetic proteins (BMPs) involved in dentin began with the reports in 1967 that rabbit demineralized dentin matrics (DDM) induced bone formation in the rabbit intramuscular pockets[1,2]. Since that, there are several *in vivo* studies that the animal DDM induced ectopic bone formation in subcutaneous and intramuscular pockets in rodents[3,4]. Human DDM implant study was first reported in 1998 that allogenic intramuscular implantation of partially demineralized dentin particles failed to induce bone formation[5]. Until now, there has been no histological evidence involved in ectopic bone and cartilage induction in the published studies using human DDM particles. The purposes of this study are to confirm the capacity of hard tissue induction by the particles of completely demineralized dentin and to estimate the efficacy of the DDM as carrier matrics for the BMP-2 delivery system

Materials and Methods

Preparations of DDM

Adult, human third molar teeth were donated by outpatients at Health Sciences University of Hokkaido. The extracted third molar teeth were collected and prepared for completely demineralized dentin matrix (DDM) as reported previously [6]. Briefly, the teeth were crashed in liquid nitrogen, washed in 1M sodium chloride, and demineralized completely in HCl solution (pH2.0) for DDM. The DDM particles were extensively rinsed in cold distilled water and lyophilized. The particle sizes varied from 0.4 to 0.8 mm (Fig. 1).

Human DDM alone implant in nude mice

Nude mice (male, 4 week-old) were subjected to intraperitoneal anesthesia with pentobarbital sodium (4 mg/ 100 g body weight). Two subcutaneous pockets were prepared in the back skin. After implantation, the incisions were sutured with nylon threads. The implanted materials were removed at 4 weeks.

Composition of BMP-2 solution and DDM

One hundred micro-liter of recombinant human BMP-2 (50μg/ ml PBS, supplied by Yamanouchi Pharmaceutical Co Ltd, Japan) solution was mixed with 70 mg of DDM in a cut-opened tuberculine syringe (Fig. 2).

BMP-2/DDM implant in rats

Operations in Wistar rats (male, 4 week-old) were done in the same manner. The implanted materials were removed at 4, 8, 32 weeks after implantation. All procedures were followed the Guidelines in Health Sciences University of Hokkaido for Experiments on Animals.

Morphological examinations

The specimens were fixed in 10% neutral buffered formalin, decalcified with 10% formic acid, embedded in paraffin, sectioned and stained with hematoxylin and eosin (HE). For morphometric analysis, tissues of the implant were divided into four compartments: bone, marrow, DDM and connective tissue.

Results

The DDM alone induced bone and cartilage in nude mice, independently (Fig. 3). Time-course study of BMP-2/DDM in rats was summarized in table 1. The BMP/DDM implant induced bone

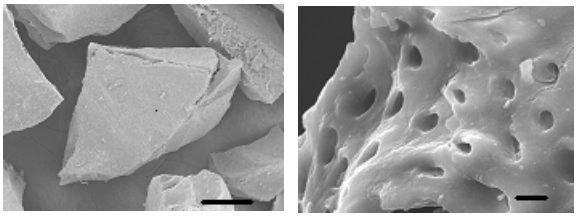

Fig. 1. SEM photographs of DDM particles
A: bar = 100μm, B: bar = 1μm,

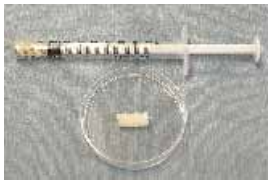

Fig. 2. Cut-opened syringe including DDM (70mg). Note DDM pellet in dish.

(Fig. 4), while the DDM alone in the rat ectopic model did not induce bone and cartilage.

Discussion

There are no reports that calcified dentin matrics (DM) can induce bone or cartilage. Lack of inductive properties of the calcified DM may be related to the inhibition of BMP-release by apatite crystal, the dense tube-structure, the quantity of dentin BMPs, and the inactivity of BMPs by procedures of the DM production. We confirmed that completely demineralized, human DM including small patches of cementum induced bone and cartilage independently in subcutaneous tissues of nude mice. However, it is possible that human DDM of vital teeth origin is less osteoinductive than DDM derived from short-lived animals such as rats, rabbits, mice, pigs and cattles [1-5,7,8]. In this study, independent phenomenon of bone and cartilage formation by the DDM alone implant was compatible to our previous report using bone-derived BMPs [9]. In 1991, a protein with BMP-like activity was isolated from human dentin, but the NH_2-terminal amino acid sequence did not resemble that of the known BMP family[10]. Moreover, amelogenin-polypeptides with chondrogenic/osteogenic-inducing activity were isolated from normally processed bovine dentin (5 month-old) in 1999, but unrelated to the BMP family[11]. The presence of BMP molecules in dentin, therefore, has been unclear until now. We believe that BMP-like molecules and amelogenin-polypeptides might be synergistic factors involved in chondrogenic/osteogenic capability of dentin.

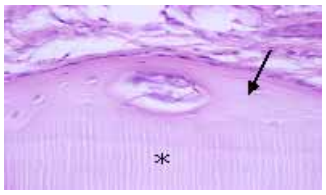

Fig.3 Induced bone (↑) connected with DDM (*) (HE x400).

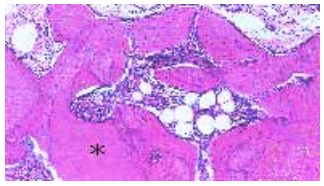

Fig. 4. Bony bridge (↑) between DDM (*) particles (HE x100).

Table 1. Changes in percentage portion of each tissue

	Weeks after implantation		
	4	8	32
Bone	28.3 ± 2.62	33.3 ± 0.47	38.0 ± 1.41
Marrow	8.0 ± 2.94	33.6 ± 2.35	41.0 ± 1.63
DDM	34.4 ± 2.86	32.4 ± 3.09	21.0 ± 2.62
C T	29.3 ± 3.29	0.7 ± 0.94	0

Total volumes = 100%, CT: connective tissues, values = S.D, numbers = 9

Conclusion

Human DDM particles are osteoinductive, insoluble collagenous matrics and it is possible that the DDM are effective as a carrier of BMP-2 for bone engineering.

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