Clinical Report

Tooth Bank System for Bone Regeneration - Safety Report -

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Abstract: The use of auto-tooth bone grafts fabricated from patients' own extracted teeth has become possible due to the development of tooth banking procedures. The Korea Tooth Bank (KTB), established in Seoul in 2009, is one such tooth-banking facility that can procure and store teeth, and then process them into bone graft substitutes. Another is the Hospital Tooth Bank (HTB) at Seoul National University Bundang Hospital (SNUBH), established in 2010 for performing storage and grafting of auto-tooth bone grafts based on experimental and clinical research. Extracted teeth are sent to the above-mentioned facility units and then delivered back to the patients for clinical use, and thus the safety of the auto-tooth bone graft materials as well as the clinical effectiveness must be guaranteed through proper quality assurance (QA) procedures. For the purpose of this investigation, we analyzed written documents for QA at KTB, and we performed histopathologic and microbiologic examinations against the banked tooth materials at the HTB. The results suggest that the tooth banking systems at both KTB and HTB sufficiently ensure patient safety.

Key words: Tooth bank, Auto-tooth, Bone graft, Tooth, Bone

Introduction

Autologous bone has been considered a gold standard for the regeneration of bone defect, however, it has disadvantages of not only the limited amount, but also donor site morbidity and etc. To overcome these limitations, much effort has continued to develop more ideal bone grafting materials¹⁾. Based on many dentin reports, we have been interested in both the osteoinduction capacities of dentin and the possibilities of bio-recycling of teeth which considered as medical waste for a long dental history¹⁻¹¹⁾. The use of autotooth bone graft materials, which are made from patient's own extracted teeth, is nowadays a standard dental procedure in Korea²⁾. While a first clinical report of human bone autograft was done in 1820, dentin autograft for sinus lifting was achieved in 2002, using a patient's own DDM granules, and reported in 81st IADR, 2003⁴⁾. It is widely used for repair of alveolar bone defects in cases of simple bone graft, guided bone regeneration (GBR) as well as sinus bone graft^{1,12-18)}. We began to use the tooth which were transformed into either the powder type or the block type to

be processed as demineralized dentin matrix under the patent "on method for processing a bone graft material using teeth and a bone graft material manufactured thereby" on October 31, 2012 (No: 10-1198115) and "on block membrane implants using autologous tooth and a machining method of the same capable of improving stability of implant surgical operation" August 30, 2011 (No: 10-1062381)^{19,20)} (Fig. 1). So that in 2009, Korea Tooth Bank (KTB) which is performing transformatin of tooth into bone graft materials and Hospital Tooth Bank (HTB) in Seoul National University Bundang Hospital performing storage and grafting of bone graft materials from patient-own teeth are established in accordance with the definition of Tooth Bank which is deemed to exist that human teeth from a dentist are collected, stored, processed into graft material for alveolar bone repair²¹⁾. Even though Korea FDA defined this item as medical services only in case of using for the patient's own as well as we had clinical evidences of effectiveness for this novel biomaterials, the safety of processing procedures is of paramount important and quality assurance is inevitable for human clinical trials because this is human tissues not categorized at this moment.

The aim of this report is to investigate the quality assurance of

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Table 1. Summary of Test Results.

Sample	List	Range	Average
AutoBT (Powder type)	R.W.C	2.8~3.8%	3.40%
	R.R	4~19ppm	7.25ppm
	ECH	0~1mg/kg	0.25mg/kg
	ETO	0mg/kg	0mg/kg
Root Form(Block type)	R.W.C	6.76~17.40%	10.80%
	R.R	0~48ppm	14.5ppm
	ECH	0~212mg/kg	53mg/kg
	ETO	0~24mg/kg	6mg/kg

Data analysis every 3 months (average) for tooth powder and block after processing. RWC: remained water content, RR: remained reagent, ECH: ethylene chloride, ETO: ethylene oxide.

tooth banking in KTB and HTB. In order to investigation, we conducted an audit of both tooth banks, which at this time involved an intern evaluation of the documents of quality assurance for KTB procedure as well as histopathologic and microbiologic examinations of banked materials in HTB.

Materials and Methods

Audit of Korea Tooth Bank (KTB)

KTB had processed 38,702 teeth as tooth powders and blocks and delivered to dental clinics for grafts (Jan. 2009 - Oct. 2012). In this audit, we investigated the written documents of screening test every quarter for the quality control such as the particle test on facility to keep Class 10,000 as well as measurements of remained reagent after processing, i.e. demineralization, defatting, sterilization and remained water content after freeze drying on the processed tooth. In addition, documents of the microbiologic test were conducted on random samples of tooth materials after the processing. For non-contamination, each tooth should be enrolled into the process separately and independently. Microbiologic culture test on tooth materials after processing are also performed and documented as a tool for quality control and assurance according to Technical Manual of Korea Tooth Bank⁽²¹⁾.

Audit of Hospital Tooth Bank (HTB) in Seoul National University Bundang Hospital (SNUBH)

Figure 1. Extracted tooth and autotooth after processing.
a. Tooth after extraction.
b. Powder after processing.
c. Block after processing.

Figure 2. SEM of fresh and old DDM powder.
a, b. Fresh DDM. Clean spaces of dentinal tubules and loosened collagen around tubules just after demineralization.
c, d. Old DDM after 2 year 9 months banking. Clean, enlarged dentinal tubule and loosened collagen around tubules and matrix.
Note: No obstructions or narrowing of tubules and no gelatination or denaturation of collagen fibers.

Totally, 476 autotooth bone grafts in HTB were returned from KTB and stored for the purpose of bone grafting during same period. Even though the majority of cases of operation have been done within one month after getting back the tooth as bone graft substitutes, some of them were banked for longer period according to the patient's need. Among the banked autotooth bone grafts, we selected 5 patient's samples with written informed consent to go through ultrastructural examination using scanning electron microscopes (SEM) to evaluate the collagen after long-term storage in HTB and through the microbiologic culture test to evaluate the maintenance of sterility.

These operations and the publication of this clinical report were approved by KTB and HTB in SNUBH.

Table 2. Culture Test Result for Sterility after Processing.

TEST RESULTS				
Test Item	Unit	Sample	Result	Test Method
Drying Loss	%	-	9.5	KS M 0009: 2010
Total Aerobic Bacterial Count	CFU/g	-	< 10	Notice of Korea Food and Drug Administration No.2012-1
Total Anaerobic Bacterial Count	CFU/g	-	< 10	By The Client(Notice of Korea Food and Drug Administration No. 2012-1)

Preparation of the sample: Sample 1 g/ Saline 9 ml, stomacher 60 sec, room temperature.
USAGE: QUALITY CONTROL.

Figure 3. Case of vertical and horizontal bone defect (57y, male).
 a. Installed implant at septal bone to get primary stability.
 b. DDM powders applied to repair the vertical and horizontal defect. See the wettability of the powders with patient-own blood.
 c. Implant fixture and cover screw covered completely with newly formed bone at 4 months.
 d. Removed bone over the cover screw for histologic evaluation.

Figure 4. Histological evaluation of removed tissue.
 a. Gross image.
 b. Lamellar bone structure (HE).
 c. DDM particle showing direct contact to new bone with viable osteocytes. Bone marrow with rich vascular supply (HE).
 d. Absorption of DDM supported by vascularized fibrous tissues (HE).

Table 3. Particle test of the facility for air contamination.

Clean Room Particle Test											
1. R/M Name				Korea Tooth Bank Co., LTD.				2. Test Date		3.10.2014	
3. Standard(EA/CFM)				10,000				4. Tested By		Air Vision Co., LTD.	
Test Point No.				Test Point No. 1				Test Point No. 2			
DATE	03/10/11	TIME	09:13:03	DATE	03/10/11	TIME	09:16:47	DATE	03/10/11	TIME	09:18:00
LOCATION	32	PERIOD	00:01:00	LOCATION	32	PERIOD	00:01:00	LOCATION	32	PERIOD	00:01:00
Size	Cumulative	Differential		Size	Cumulative	Differential		Size	Cumulative	Differential	
0.3μm	0	0		0.3μm	87	77		0.3μm	111	104	
0.5μm	0	0		0.5μm	10	7		0.5μm	7	6	
0.7μm	0	0		0.7μm	3	2		0.7μm	1	1	
1.0μm	0	0		2.0μm	1	1		1.0 μm	0	0	
5.0μm	0	0		5.0μm	0	0		5.0μm	0	0	
Test Result 0.5f μm (EA/CFM)				Test Result 0.5f μm (EA/CFM)				Test Result 0.5f μm (EA/CFM)			
Particle 0.3μm		0		Particle 0.3μm		870		Particle 0.3μm		1,110	
Particle 0.5μm		0		Particle 0.5μm		100		Particle 0.5μm		70	
Judgment		PASS		Judgment		PASS		Judgment		PASS	

Judgment declared pass or fail.

Results

Audit of KTB

Measurements of remained water content, remained reagents

were summarized in Table 1. Remained water content after freeze-drying of powder and block was average 3.40 %, 10.8 % respectively. Average of remained reagent became higher than

usual due to accidental error of processing in one event 19 ppm and 48 ppm in cases of powder and block. Example sheet of microbiologic culture test for sterility, measurements of remained water contents and remained reagent test against the grafts materials after processing was in Table 2. Microbiologic culture tests were all within normal limits. Example sheet of particle tests on the facility was in table 3, showing acceptable micro-dust to keep Class 10,000 in the air of facility.

Audit of HTB in SNUBH

Ultrastructural examinations using SEM were performed on powder immediately after processing, so called fresh DDM, to compare with the banked DDM thereafter (Fig. 2a). The SEM photo showed clean dentinal tubules and loosened collagen around tubules and matrix after demineralization. Powders of 5 patients (average: 25 months, banking period: 4 months to 3 years) were also examined to compare with fresh DDM materials. The picture showed clean, enlarged dentinal tubule and loosened collagen around tubules and matrix after 2 year 9 months banking (Fig. 2b). There was no obstructions or narrowing of tubules and no gelatination or denaturation of dentin collagen fibers in the matrix (Fig. 2b).

Discussion

Characterization of autotooth bone graft materials

The chemical compositions of dentin and bone resemble each other. The alveolar bone has 61 % of inorganic, 32 % of organic substances and water, while the dentin has 65 % of inorganic substances, 29 % of organic substances and water. The cementum is composed of 45-50 % of inorganic substances, 50-55 % organic substances and water²²). Based on inorganic component analysis by Kim et al, autogenous tooth bone graft material consists of 5 biological calcium phosphates (low crystalline hydroxyapatite and another calcium phosphate minerals such as TCP, amorphous calcium phosphate, dicalcium phosphate dehydrate, octacalcium phosphate). These calcium phosphates interact reciprocally and are capable of remodeling the existing bone when grafted. Therefore, the more less crystalline the carbonic apatite which exist in bone and tooth is, the more osteoconductive the graft material is when used as a bone graft material^{23,24}). Regarding organics of this demineralized dentin matrix (DDM), there are type I collagen, and various growth factors including BMPs, which induce bone and cartilage, and non-collagenous proteins such as osteocalcin and osteonectin, which have been implicated in calcification. Dentin-specific proteins including phosphoprotein, (phosphophoryn) and dentin sialoprotein have also been identified in dentin matrix²⁵). It is well known that appropriate demineralization process does not denature the type I collagens, growth factors, and non-collagenous proteins so that the DDM can maintain osteoinductive-healing capacities^{5-7,18,25}).

Demineralized dentin implanted subcutaneously in the dorsal region of rats not only induced infiltration of monocytes and development of foreign body giant cells, but also induced chondrogenesis in vitro⁷). Gelatin capsules containing 70 mg of demineralized human dentin particles were implanted into the femoral muscles of nude mice, and induced a slight formation of bone and cartilage²⁶). These studies indicate that dentin contains bone and cartilage-inducing factors that facilitate chondro-osteogenesis and modify the calcification process.

Clinical reports

Kim and coworkers reported, in 6 patients of guided bone regeneration (GBR) with implant operation, that DDM powder underwent gradual resorption and was replaced by newly formed bone with well vascularization¹⁴). Park and coworkers also reported that there were no infections among 250 patients who received autotooth bone grafts with GBR, sinus grafts, socket preservation and ridge augmentation from Sep. 2009 to Dec. 2011¹⁶). Tazaki and coworkers performed an immediate DDM graft around a transplanted tooth. The dental X-ray showed periodontal space and lamina dura at 12 months after the operation. They suggested that the preserved autogenous DDM could be used as collagenous biomaterials with osteoinductive potency²⁷). According to the retrospective cohort study from 2008 to 2009, 37 patients who received sinus bone graft, ridge augmentation and GBR with powder showed minimal to no post-operative complications during 2-year follow up. The complications that they found were the wound dehiscence, hematoma and failure of osseointegrations, which are not related to the DDM materials¹⁷).

Case of vertical and horizontal bone defect

A left lower first molar (#36) of 57 year-old male patient that was stored in refrigerator within the neutral alcohol, was sent to the KTB processing facility. After removing all the foreign materials such as restorations, soft tissues, blood, and root canals from the tooth, tooth-powders crushed by using mills were sieved from 400 to 800µm. Then, demineralization, defatting, freeze-drying, sterilization and packaging under Guidelines were implemented in the aseptic condition and individual lot (Fig. 1). After installation of implant in septal area to get primary stability, the vertical and horizontal defects of sockets were augmented with prepared DDM powders simultaneously (Fig. 3a,b). Complete bone formation was found around the implant fixture at 4 months after the graft (Fig. 3c). A hard tissue over the cover screw was taken for histologic evaluations (Figs. 3d, 4a). Almost all DDM were turned into mature bone mixed with a few remained particles which showed very close contact with newly formed bone as well as showed resorption interpreted as remodeling (Fig. 4b-d).

Quality assurance of KTB

The amount of remnant H₂O in powder form (Fig. 1b) is 2.8 %-3.8 % (average: 3.4 %) in that storing at room temperature (Table 1). Block form (Fig. 1c) also suits storing at room temperature with remnant H₂O amount being 6.7 %-17.4 % (average: 10.8 %). The amounts of remnant EO and ECH were detected within normal range except twice above normal values, yet no eventful issues were found after adjustment. Microorganisms after culture of powder and blocks appeared with normal ranges (Table 2). Particle test for air contamination of processing facility in every 3 months revealed within normal limits to keep class 1,000 processing facility at this audit (Table 3). The *in vitro* experiment to evaluate the biocompatibility and safety of this powder with cell adhesion and differentiation test supported that tooth powder after processing was both biocompatible and osteogenic (unpublished data).

Ultrastructural and microbiologic examinations of HTB in SNUBH

There were no abnormalities on the dentin matrix after 2 year 9 months banking (Fig. 2c,d), compared with the fresh normal dentin particles after processing in that sound dentinal tubules and intact collagen fibers (Fig. 2a,b). This suggested that the remained water content of powder and block was suitable for the long term banking of materials at room temperature. It may also be possible, therefore, to cryopreserve DDM obtained from extracted teeth using liquid nitrogen, so that the DDM can be stored for longer than 10 years. Bacteria-free of the DDM was confirmed by the bacteriological examination using blood-agar medium culture²⁸).

In conclusion, extracted tooth provides a useful source of bone graft materials by the development of tooth banking technology. Many clinical studies and reports supported the effectiveness of autotooth bone grafts in the dentoalveolar bone repair. We can conclude that the banking procedures for DDM in both KTB and HTB were safe and gave us the valuable information of improvements to be made. We can also suggest that even if there is no uniform guidelines or standards for management of tooth bank yet, KTB and HTB in SNUBH have been trying to develop the standard system for more effective and safe tooth banking in many countries.

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