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Original Article

Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy Studies on Processed Tooth Graft Material by Vacuum-ultrasonic Acceleration

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Abstract

Purpose: The current gold standard for clinical jawbone formation involves autogenous bone as a graft material. In addition, demineralized dentin can be an effective graft material. Although demineralized dentin readily induces heterotopic bone formation, conventional decalcification takes three to five days, so, immediate bone grafting after extraction is impossible. This study evaluated the effect of vacuum ultrasonic power on the demineralization and processing of autogenous tooth material and documented the clinical results of rapidly processed autogenous demineralized dentin (ADD) in an alveolar defects patient.

Methods: The method involves the demineralization of extracted teeth with detached soft tissues and pulp in 0.6 N HCl for 90 minutes using a heat controlled vacuum-ultrasonic accelerator. The characteristics of processed teeth were evaluated by scanning electron microscopy (SEM), and energy dispersive X-ray spectroscopy (EDS). Bone grafting using ADD was performed for narrow ridges augmentation in the mandibular area.

Results: The new processing method was completed within two hours regardless of form (powder or block). EDS and SEM uniformly demineralized autotooth biomaterial. After six months, bone remodeling was observed in augmented sites and histological examination showed that ADD particles were well united with new bone. No unusual complications were encountered.

Conclusion: This study demonstrates the possibility of preparing autogenous tooth graft materials within two hours, allowing immediate one-day grafting after extraction.

Key words: Autogenous, Demineralized dentin, Vacuum, Ultrasonic, Energy dispersive X-ray spectroscopy

Introduction

Autogenous bone grafts are extensively used to augment severely resorbed alveolar ridges, although other materials, such as, undecalcified, allogeneic devitalized bone, frozen bone, processed xenogeneic bone, deproteinized bone, and hydroxyapatite (HA) crystals, are also used for bone regeneration. Dentin is a potentially valuable graft material. Initially, particulate dentin (tooth ash) was used as a graft material, because hydroxyapatite is the main ingredient of teeth. Because it is bioresorbable, particulate tooth ash can also be used as an alternative graft material for im-

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plants[1]. Demineralized dentin has recently been used for the reconstruction of alveolar bone[2]. In fact, the chemical composition of dentin is similar to that of bone. It is composed of a hydrated organic matrix consisting mainly of type I collagen and an inorganic reinforcing phase of carbonated apatite. Dentin is composed of inorganic (70%～75%), organics (20%), and water (10%), whereas in alveolar these percentages are 65%, 25%, and 10%, respectively[3]. On the other hand, decalcified dentin is a composite matrix of type I collagen and growth factors. Furthermore, dentin and bone demineralization increases osteoinductivity, and decreases antigenicity. The bone-inducing property of dentin was discovered in 1967 when demineralized dentin matrices (DDM) of rabbit were found to induce bone formation in intramuscular tissues[4].

A problem with demineralized dentin as a graft material is the time it takes to decalcify and produce as a bone graft material from teeth. In particular, tooth blocks require more processing time than tooth chips or powder. The conventional method requires 10 days or more to complete because bone transplant material must be prepared using extracted teeth. This requires transport to a processing company, and at least 10 hours is required to demineralize a tooth block. As a faster alternative method, we developed a vacuum-ultrasonic accelerator (VacuaSonic™; Cosmobiomedicare, Seoul, Korea) based method that can be performed in clinics. The whole process can be completed within two hours regardless of the form (powder, chip or block) used.

The new method utilizes ultrasonication and a vacuum. Ultrasonication uses for cleaning, but because of the hardness of teeth, it is difficult to clean hidden surfaces (dentinal tubules). In addition, it produces trapped air bubbles in dentinal tubules. We used a periodic vacuum system and specific ultrasonic frequency to solve these problems. The chemical treatment employed with ultrasonication included a vacuum, which noticeably improved efficiency, and markedly reduced overall treatment time[5].

We studied the ultra-structure and chemical components of newly processed tooth graft material by scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS). A clinical case revealed the ability of the graft materials produced to regenerated bone after implant surgery. Here we report preliminary results obtained from our experimental studies and a clinical case of alveolar bone regeneration using the novel tooth grafts.

Materials and Methods

1. Materials

Chungbuk National Hospital BioBank provided tooth samples (n=8) for this research, and all donors provided informed consent and the study was approved by IRB. The samples were randomly allocated to an autogenous demineralized dentin (ADD) group (n=5) and an untreated group (n=3). Human extracted teeth were washed in a solution of 0.9% NaCl after soft tissue and pulp removal and stored at −70°C.

In the ADD group, remnant tissues around the roots of extracted teeth were removed using a surgical blade or periosteal elevator. A dental handpiece was used to remove pulp tissue from the pulp chamber and root canals and perfuse the reagent effectively. In addition 0.3 mm diameter holes were made along the entire tooth surface using a 330 bur (Fig. 1). The tooth was placed in a sterile solution for 10 minutes in the vacuum ultrasonic system. Teeth were demineralized in a 0.6 N solution of HCl for 90 minutes in the vacuum ultrasonic machine (VacuaSonic™), and washed for twice for 15 minutes in deionized sterile water or phosphate buffered saline (PBS) solution. The entire processing time was less than 2 hours (Fig. 2). After removing the PBS solution, processed teeth graft materials were packaged into a sterile tube.

In the untreated group, remnant tissues around the roots were removed using a surgical blade or periosteal elevator,

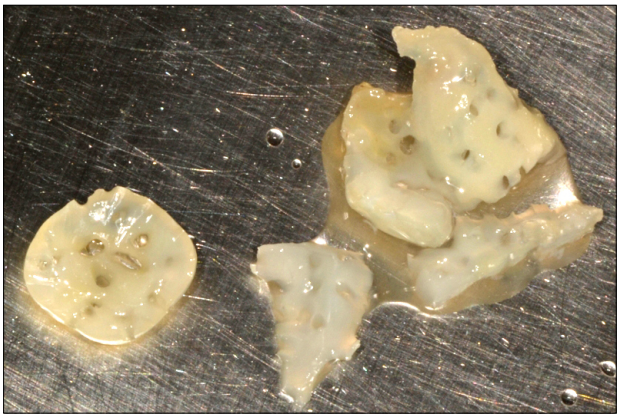

Fig. 1. Processed teeth (autogenous demineralized dentin).

then the teeth were washed in a saline solution.

Two groups of tooth samples were prepared in the form of 2 mm cubes for SEM and EDS studies. Cubes were removed from the tooth necks with a #15 blade (ADD) and a high speed bur (untreated teeth).

2. Measurements of wet tooth weight

To quantify weight reduction, weights were measured before and after processing in ADD group.

3. Scanning electron microscopy

Observations of ADD material and untreated tooth surfaces were performed optically using a scanning electron microscope, in secondary electron mode. SEM studies were performed using a Nanoscope IIIa instrument (Digital Instruments, Tonawanda, NY, USA), after coating samples with a gold-palladium alloy.

4. Energy dispersive X-ray spectroscopy

To determine whether a change in mineral components occurred during processing, EDS was performed using the TEAM™ system (Bruker Co., Ewing, NJ, USA). To determine internal mineral concentrations, processed tooth materials and unprocessed teeth were checked for calcium concentration (wt%) at a depth of 300 to 600 micrometers from tooth surface.

5. Clinical case

A 45-year-old male patient visited Chungbuk National University Hospital for extraction of #48 tooth. Radiological and clinical examinations revealed an impacted third molar, a periapical and a periodontal lesion of #47, and an edentulous alveolar ridge on #44 with narrow ridge (Fig. 3).

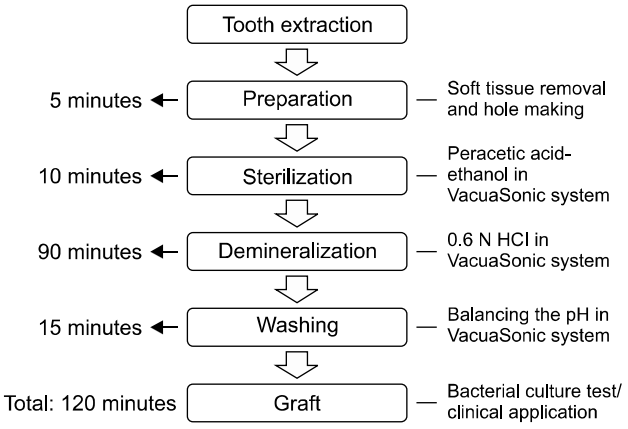

Fig. 2. Autogenous demineralized dentin processing procedures (from extraction to graft).

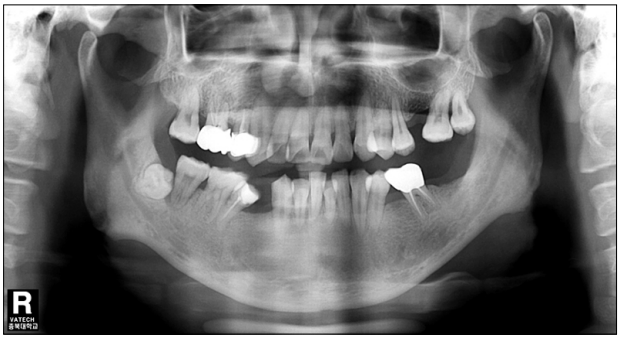

Fig. 3. Panoramic view; pre-extraction of #47, 48.

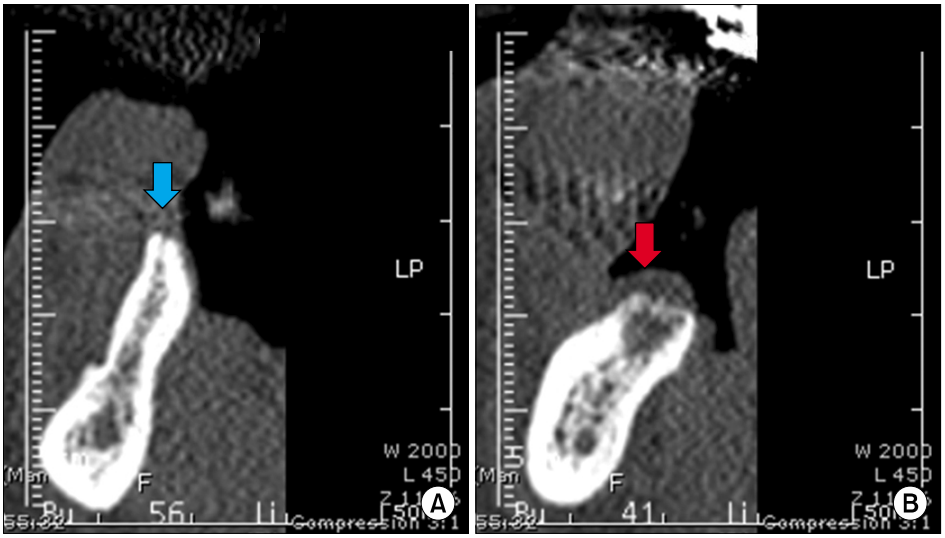

Fig. 4. Pre-operative computerized tomography. (A) #44 extracted area (arrow: narrow ridge). (B) #47 extracted area (arrow: narrow ridge).

Therefore, surgical extraction of #47 and 48 and implant placement on #44 and 47 with ADD were planned. #47 and 48 teeth were extracted before surgery, and block-type autogenous tooth graft materials were produced in a clinic. For personal reasons, surgery was delayed for six months, and processed materials were stored at −70°C in a freezer. Preoperative computer tomography was performed (Fig. 4). The patient was prepared and sterilely draped for intraoral surgery. Significant narrow ridges on #44 (2 mm) and #47 (4 mm) edentulous sites were noted. Implants were placed on the #44 (diameter 4.0 mm, length of 10 mm; TSIII; Osstem, Seoul, Korea) and #47 area diameter 4.5 mm, length of 10 mm; TSIII). Exposed implant threads were noted due to the narrow ridges (Fig. 5). An autogenous tooth chip was prepared with the bone mill and placed on the buccal side of alveolar ridge. To increase volume, a block type of ADD was transplanted upwards autogenous tooth chip with resorbable collagen membrane (CyADDlast; Osteogenic Biomedical Inc., Lubbock, TX, USA) (Fig. 6). After suturing without tension, antibiotics and anti-inflammatory analgesic were administered and the patient gargled with saline.

Results

1. Wet tooth weights

The weights of teeth before processing were measured at an average 0.38 g (range, 0.23∼0.53 g). The weights of teeth after processing averaged 0.20 g (range, 0.13∼0.32 g), 43.4% average reduction (Table 1).

2. Scanning electron microscopy

Fig. 7 shows SEM images of the processed dentin. The external aspects of ADD surfaces with open dentinal tubules were visualized. Tubule sizes increased after processing. SEM visualized processed dentin surfaces with widened dentinal tubules.

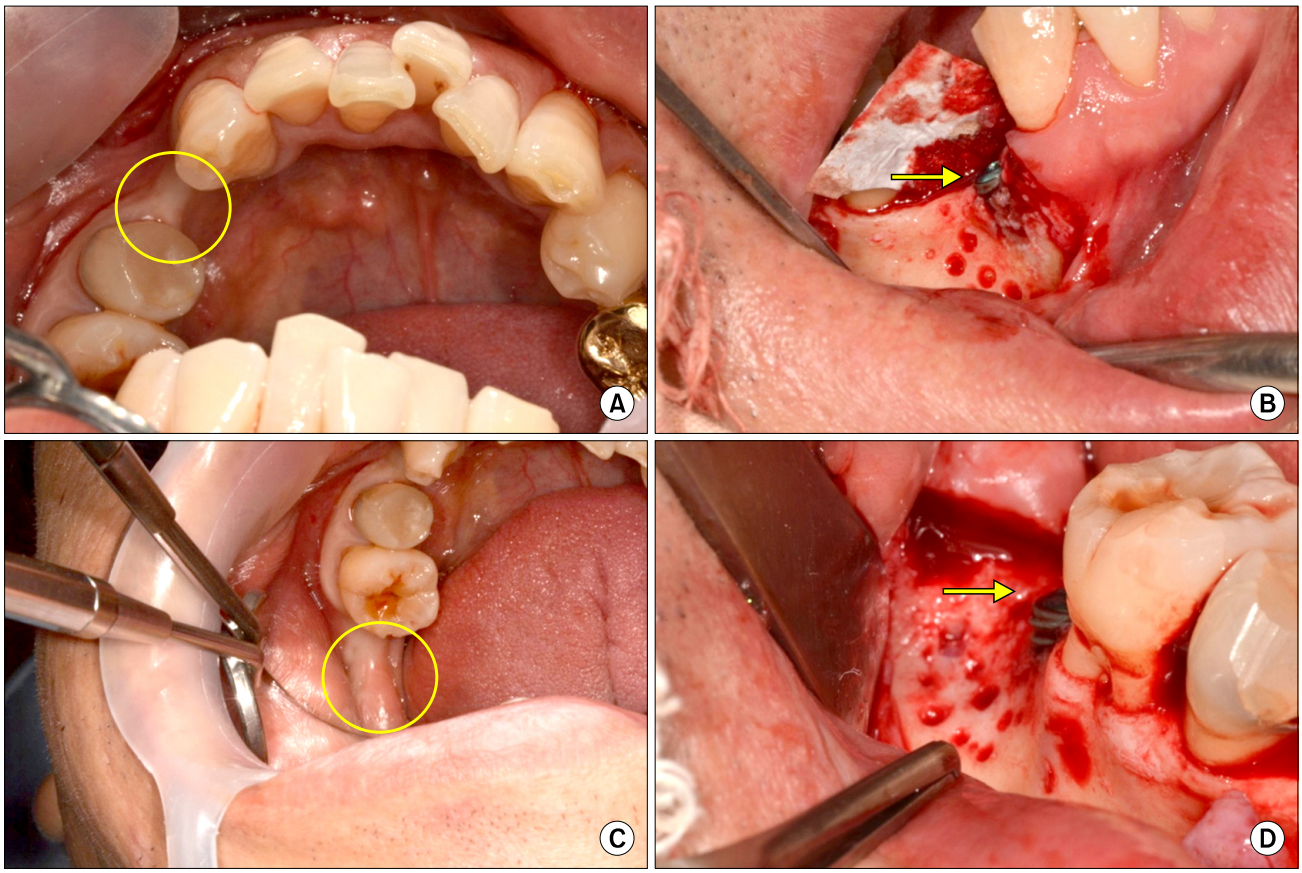

Fig. 5. Intra-operative photos. Showing are narrow ridges and exposure implant threads. (A, B) #44 extracted area (circle: narrow ridge, arrow: exposed fixture). (C, D) #47 extracted area (circle: narrow ridge, arrow: exposed fixture).

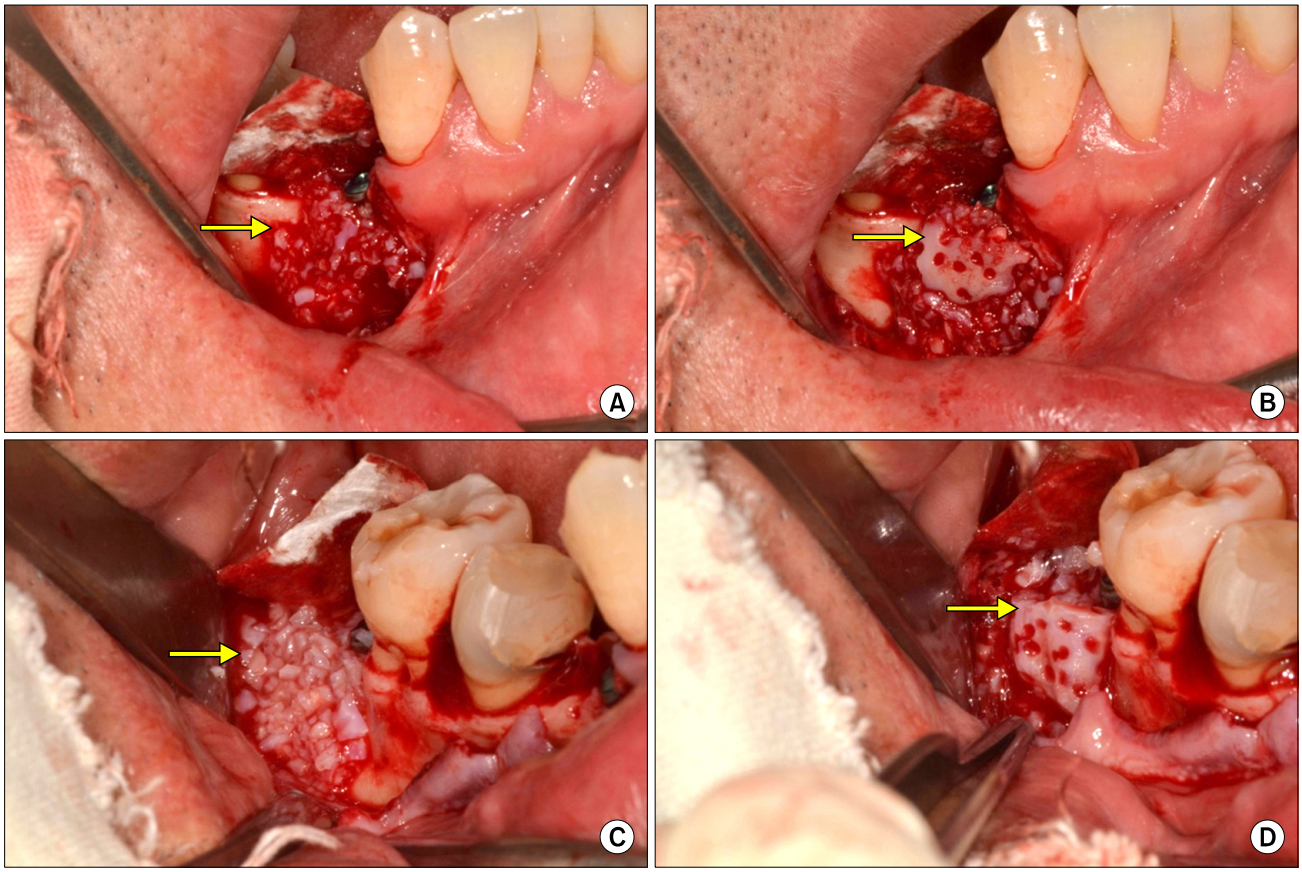

Fig. 6. Autogenous demineralized dentin chip and block graft. (A) #44 extracted area (arrow: particulated processed tooth graft). (B) #44 extracted area (arrow: block processed tooth graft). (C) #47 extracted area (arrow: particulated processed tooth graft). (D) #47 extracted area (arrow: block processed tooth graft).

Table 1. Autogenous demineralized dentin weight measurements

Patient No.	Donor age (yr)/sex	Pre-processing (g)	Post-processing (g/%)
1	52/male	0.53	0.32/60
2	40/male	0.28	0.16/57
3	18/female	0.51	0.26/51
4	22/male	0.23	0.13/57
5	56/male	0.35	0.20/58

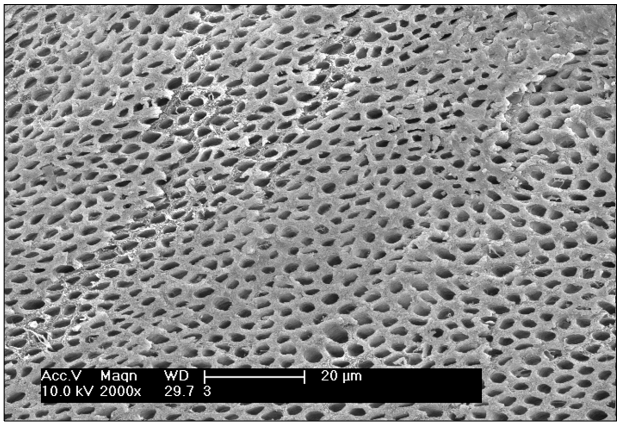

Fig. 7. Scanning electron micrograph of a dentin surface after the Autogenous demineralized dentin processing (bar scale 20 μm).

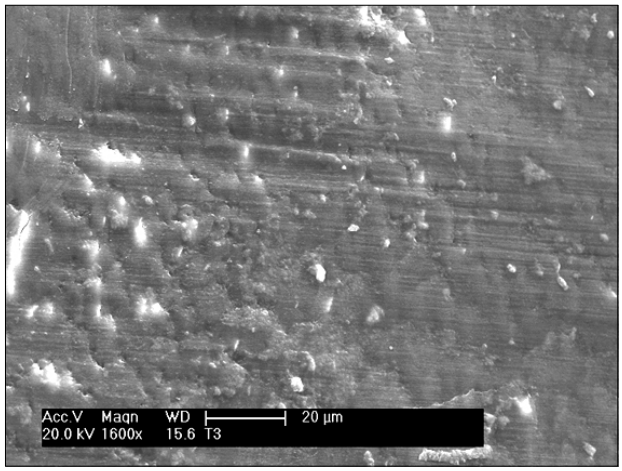

Fig. 8. Scanning electron micrograph of an untreated dentin surface (bar scale 20 μm).

We paid attention to unprocessed dentin on the surfaces. Dentinal tubules were not seen. Fig. 8 shows a dentin surface with a smear layer and ground tooth particles produced by a high speed bur. In untreated teeth smear layers were composed of small particles of dentin and dendrites

covered dentin surface.

3. Elemental analysis by energy dispersive X-ray spectroscopy

The calcium and phosphate levels of untreated teeth were higher than those of ADD. Mineral concentrations, were significantly lower for ADD. Changes in calcium level in ADD grafts were observed. The calcium concentration of ADD was 6.52 wt% whereas that of normal dentin was 31.17 wt% at a depth of 300~600 micrometers from external surfaces (Table 2), indicating that 79% of the calcium had been removed (Fig. 9, 10).

Table 2. Calcium concentration of processed (autogenous demineralized dentin) and unprocessed teeth

Variable	Depth (μm)	Calcium level (wt/%)
Processed tooth (n)		
1	307.6923	7.14
2	448.7179	4.45
3	641.0256	5.24
4	384.6154	7.42
5	551.2821	8.34
Unprocessed tooth (n)		
1	411.9318	30.98
2	553.9773	32.46
3	696.0227	30.07

4. Clinical observations and histopathologic analysis

In the described case, healing was uneventful. No significant complications, such as, infection, uncontrolled dehiscence, graft failure, or implant failure occurred. Six months after surgery, good bone healing and bone remodeling were observed. Further examination of computer tomographic views was performed to confirm bone healing at ADD treated defect sites (Fig. 11).

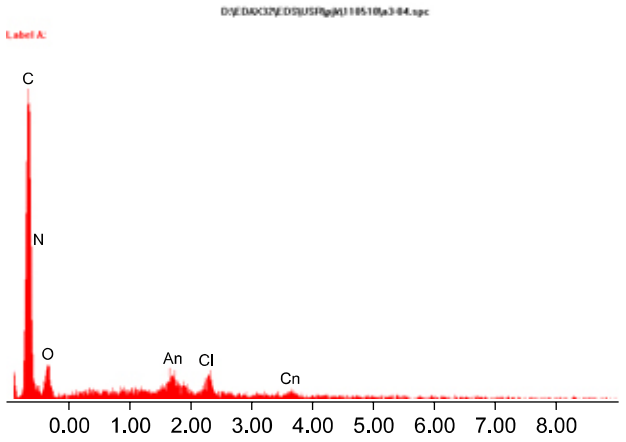

Fig. 9. Energy dispersive X-ray spectroscopy of a dentin surface after the autogenous demineralized dentin processing.

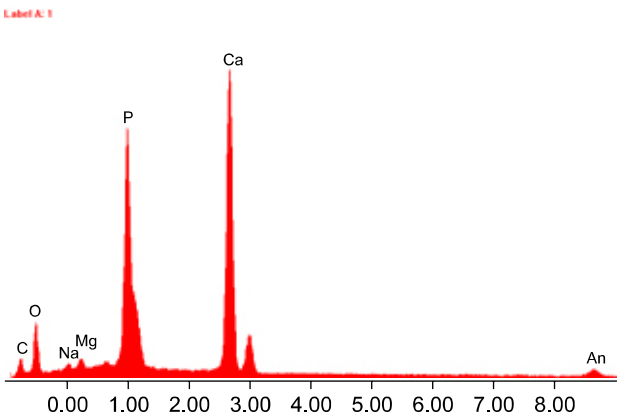

Fig. 10. Energy dispersive X-ray spectroscopy of an untreated dentin surface.

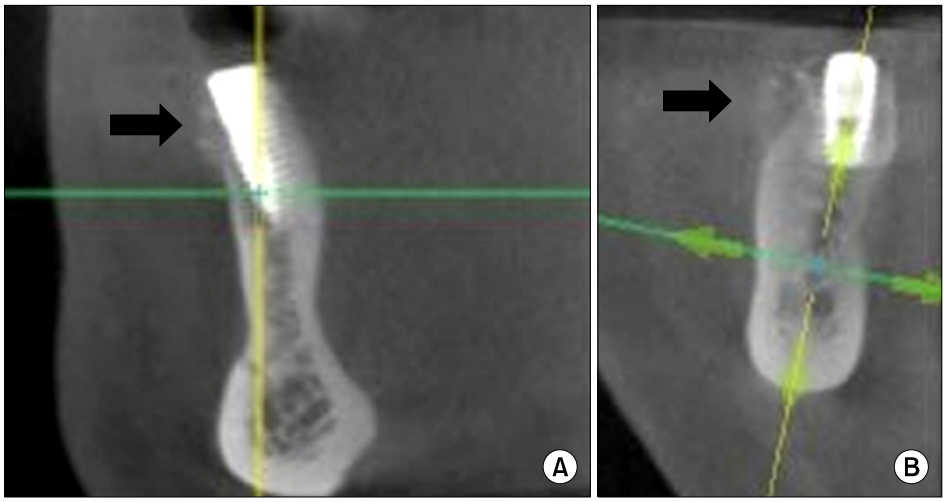

Fig. 11. Post-operative computerized tomography. Six months after the autogenous demineralized dentin graft, good bone formation is seen. (A) #44 extracted area. (B) #47 extracted area.

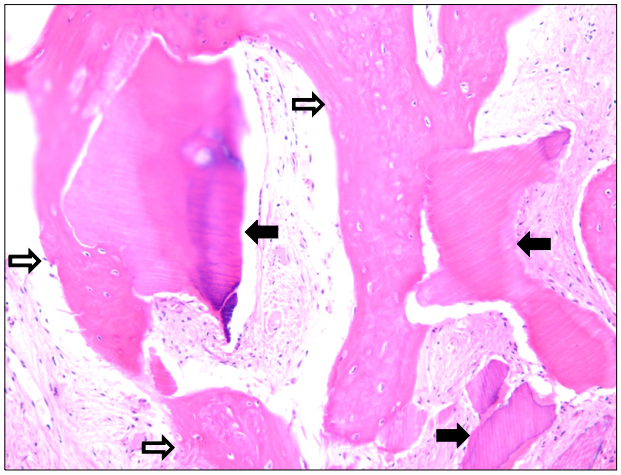

Fig. 12. Histological finding of autogenous demineralized dentin at 6 months after implantation (H&E, ×200; open arrows: new bone formation, closed arrows: processed tooth graft material). New bone formation around processed tooth material and resorption of grafted materials.

For histopathologic examination, a bone specimen was sampled at ADD treated site. The specimen showed new bone formation and bone remodeling. In addition, appositional bone growth and ADD particles resorption were observed (Fig. 12).

Discussion

The present study documented the characteristics and the ability of alveolar bone reconstruction using rapidly demineralized dentin (ADD) as a bone graft material. Dentin is an acellular collagen-rich tissue matrix without vessels, whereas bone is a cellular tissue with vessels. However, dentin and bone have similar compositions, that is, 10% body fluid, 20% organic materials, and 70% minerals (mainly HA), and contain bone morphogenetic proteins (BMPs) that have the ability to induce bone and cartilage in formation in non-skeletal sites[6,7]. The major component of the inorganic material of teeth contains several types of calcium phosphate including HA. Earlier research found that tooth ash can be used as graft material for the reconstruction of bone[1]. DDM performs better for bone induction than calcified dentin at four weeks after implantation[4]. Demineralization of dentin increases the bioavailability of matrix-associated non-collagenous proteins, rendering these grafts osteoinductive. Non-collagenous dentin proteins, such as, osteocalcin, osteonectin, phosphoprotein, and sialoprotein are involved in bone calcification[8,9]. Furthermore, both mature and immature types of BMPs-2 are found in human dentin[10]. Decalcified dentin supports heteroinduction in experimental and clinical studies[4,11-14]. Decalcified dentine is penetrated and resorbed more slowly than decalcified bone, because it is denser and has neither old vascular channels nor marrow spaces[4]. For the same reason, the decalcification time of teeth is greater than that of bone.

Long processing times for teeth are problematic clinically. Moreover, excessive acid exposure might diminish BMP retention due to diffusional losses and graft potency. As described by Pietrzak *et al.*[15], the demineralization of bovine cortical bone using 0.5 N HCl occurred within 90 minutes and peak BMP-7 levels were reached at that point, and then fell. To overcome this problem, we developed a rapid processing method using a vacuum-ultrasonic system. Our SEM and EDS studies confirmed the effective demineralization of teeth within two hours. SEM analysis of newly decalcified dentin (ADD) revealed dentinal tubules, which we believe act as a network for the diffusion of nutrients after grafting. Type I collagen fiber accounts for 90% of the organic matrix, and is important in calcification. Demineralization of dentin and bone increases osteoinductivity and decreases antigenicity[16]. Ultrasonic treatment creates cavitation, a high energy phenomenon, inside an object by repeated compression and expansion[17]. When this cavitation is present on the surface of an object, the energy released activates a reaction between the reagent and the object. In order to use the ultrasonic system, we considered two factors; temperature and air bubbles. Increased temperature during processing affects the preservation of protein substance in graft material. As air bubbles in dentinal tubules hinders rapid demineralization, periodic vacuum application was used to prevent bubble entrapment in dentinal tubules[5].

The devised method facilitates clinical application because it can perform block processing within two hours. If the operator chooses to use chip or powder, changes can be made during bone milling. In the described case, we used block and chip (ADD) at no additional cost.

Conclusion

The described vacuum-ultrasonic system enables tooth demineralization within two hours. This study shows that the developed processing method markedly reduces decalcification times. Furthermore, our high-resolution imaging and EDS studies confirmed the decalcification of dentin. In the clinical case, we observed good bone formation in narrow alveolar ridges. Finally, we conclude rapid preparation of autogenous teeth using the devised method makes one-day grafting possible.

Acknowledgements

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