A New Method Using Autogenous Impacted Third Molars for Sinus Augmentation to Enhance Implant Treatment: Case Series with Preliminary Results of an Open, Prospective Longitudinal Study

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Purpose: *This prospective longitudinal study reports on the results in patients given autologous tooth material for augmentation in a sinus elevation procedure.* ***Materials and Methods:*** *Six patients with inadequate bone supply for augmentation in the maxillary posterior tooth region and at least one impacted maxillary third molar underwent sinus elevation surgery with lateral access using the particulate tooth material. One of the patients received four implants during the same session, while the other patients had a total of 15 implants placed after a healing phase of an average 5.5 months. Drill cylinders collected from the implant bed during the procedure were subjected to histologic/immunohistochemical evaluation.* ***Results:*** *All six patients showed normal and unobtrusive postoperative healing, having undergone prosthetic restoration up to 5 years before. The average peri-implant probing pocket depth after a period of up to 5 years ranged between 1.86 mm (mesial and lingual) and 2.07 mm (distal and buccal). No bleeding could be triggered with any of the peri-implant probes. The average peri-implant bone resorption measured during the first year was up to 0.63 mm, with the lowest being 0 mm and the maximum 2.9 mm. Peri-implant bone remained stable for the follow-up time of up to 5 years. Histologically, six biopsy specimens collected from five patients showed osteoconductive osteogenesis with encapsulation of tooth enamel and dentin portions and partial resorption of the tooth components. Cementum shares were no longer discernible. Immunohistochemical assessment showed intense new vessel formation that could be observed in the area of loose stroma of reorganized tissue in the augmented area.* ***Conclusion:*** *Within the limits of these preliminary results and with adequate consideration of the small number of patients included, the use of autogenous crushed tooth material from impacted third molars may represent an alternative augmentation material for use in sinus elevation procedures.* Int J Oral Maxillofac Implants 2016;31:622–630. doi: 10.11607/jomi.4172

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As a result of its osteogenic capacity as well as its osteoinductive and osteoconductive properties, autogenous bone is still considered as the standard for the augmentation of the atrophic implant bed.[1–3] Today, all commonly used bone substitute materials are available without limitation, and additional surgical risks may be eliminated by avoiding a second intervention. However, their use will be associated with additional costs, and apart from foreign body reactions, bone substitutes may show inadequate osteogenesis[4] or completely lack osteogenesis depending on the material selected.[5]

Considering the structural characteristics of bone, dentin, and root cementum, numerous common features can be identified.

Calcium hydroxyapatite (primarily calcium and phosphate) constitutes the primary component (70%) of these structures. Organic components (including 90% collagen type I) account for approximately 20%.[6] In addition, fluoride and a number of trace elements (Al, Br, Cl, Cr, Fe, K, Mn, Na, S, Si, Zn)[7] can also be found. The basic substance and hardness (30 to 50 KHN [Knoop Hardness]) of cementum are similar to

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that of membranous bone. It consists of 65% mineral substance (in particular, calcium and phosphate in the form of apatite crystals and amorphous calcium phosphates), an organic matrix of approximately 23%, mineralized collagenous fibers (approximately 90%), cementocytes, and 12% water. Mineral substances are deposited to approximately the same extent as in alveolar bone.[8] Tooth enamel is the hardest substance in the human organism. It contains calcium, phosphate, magnesium, fluoride, sodium, and carbonate as well as proteins and fats. Ninety-five percent of tooth enamel is inorganic and mainly consists of the acid-soluble phosphate hydroxyapatite [Ca5(PO4)3OH]. As compared with bone and dentin, tooth enamel shows a higher degree of mineralization and a significantly higher hardness, density, and crystallinity, but also a lower porosity.[9]

Tooth enamel as seen in the electron microscope consists of rod-shaped crystallites grouped in bundles and termed prisms.

Considering the congruency of dental components with bone material, it was only obvious to assess autogenous tooth material for use as autogenous augmentation material.

Animal experimental studies showed extensive bone formation in extraction alveoli of the anterior teeth of rats having been filled with ground autogenous deproteinized tooth material mixed with hydroxypropylcellulose (HPC) for 4 weeks. The mineral content of the newly formed bone was significantly higher than that in the control group given HPC alone.[10] Transmitter substances contained in tooth material such as osteopontin, a protein involved in the preservation of bone substance and in some immune processes, could provide for an inductive component of bone formation with the use of tooth material. Osteopontin binds hydroxylapatite and constitutes the basic structure for bone material.[10] Thus, the use of decalcified tooth material has been shown to induce transformation of mesenchymal stem cells into osteoblasts as evidence of osteoinduction.[11]

As yet, no animal experimental or clinical investigations using unmodified autogenous tooth material have been reported.

A case report describes the retention of a buccal socket-shield in the alveolus upon immediate implantation for preserving the esthetic profile of the alveolar process.[12] However, this material was always in periodontal connection, and for this reason, it does not represent an auto-transplantation of dental tissue. In addition, no findings for these root sections were reported.

The subject investigation was intended to evaluate the use of autogenous tooth material as augmentation material in sinus elevation procedures.

MATERIALS AND METHODS

Patients

One of the inclusion criteria for entry into this open, single-center cohort study was the presence of a terminal gap in the maxilla and a vertical bone deficit necessitating a lateral sinus elevation. An additional inclusion criterion was the presence of an impacted third molar that had not penetrated through the gingiva (Fig 1). The patient received full information on the planned procedure and had to sign an informed consent form. The study was performed according to the guidelines of the Medical University of Vienna and was approved by the local Ethics Committee (EC No. 885/2010). The study was designed as an open, single-center study.

The sinus elevation procedure was done via lateral access; the bone window was prepared with a bone scraper (Safescraper, Medos Austria) with autologous bone material being collected. The impacted molar to be used was removed and completely comminuted in a manual bone mill (Medos Austria; Figs 2a and 2b). The tooth material was mixed with the bone material from the bone scraper and applied into the subantral hollow space. If additional augmentation material was needed, bovine bone substitute material (BioOss, Geistlich) was added. In patients with an alveolar ridge height of 5 to 6 mm, implant placement was done in the course of the sinus elevation procedure, and for all other patients, it was done in a second procedure after 4 to 6 months according to the height of the original alveolar ridge. In such cases, the alveolar ridge was visualized through a mucoperiostal flap, a histologic specimen at the implant position was collected (Hollow Drill, Medos Austria; Fig 3), and further implantation was performed according to the drill protocol (Replace, Nobel Biocare; Fig 4). For both procedures, the patients were given amoxicillin 2 g as a single oral dose about 2 hours preoperatively. After another 3 months, the implants were exposed and prosthetically provided with fixed partial prostheses or single crowns.

The patients were followed at 6 and 12 months post-implantation and thereafter at annual recall intervals (Figs 5a and 5b).Clinical evaluation comprised the inspection of the peri-implant mucosa and measurement of probing depth (PD) in four different locations of the implant (labial, lingual, mesial, distal) using a periodontal probe (Hu-Friedy). The probe was invariably introduced with light pressure into the sulcus in parallel to the implant axis, and the distance from the mucosal margin to the probe tip was measured. The Bleeding Index (according to Mühlemann and Son[13]) was also measured with the periodontal probe at the four locations of the implant in the same manner.

Digital radiographs (Sidexis, Sirona) were taken at the annual follow-up visits, and mesial and distal bone

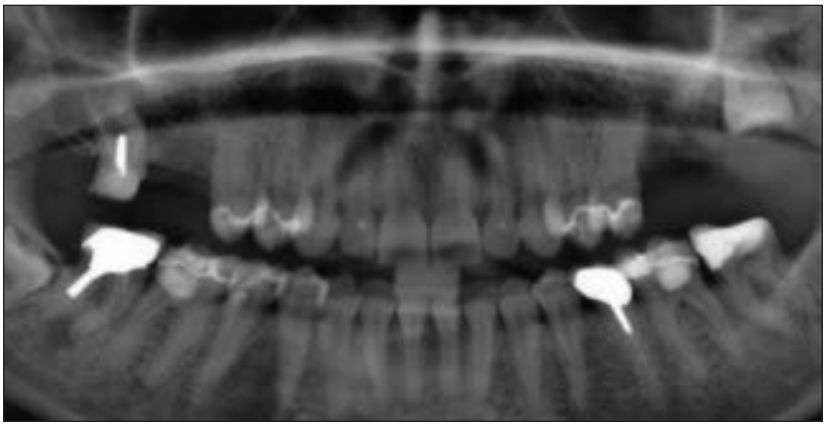


Fig 1 Preoperative orthopantogram (OPTG) showing impacted third left molar in conjunction with a terminal gap on the left side and an intermediate gap on the right side of the maxilla.

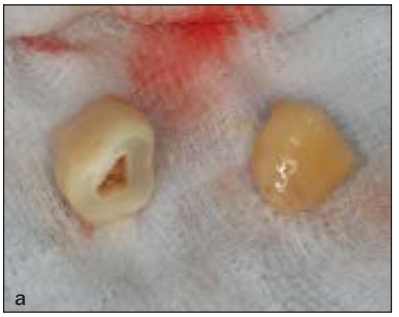


Fig 2 Removed impacted third molar *(a)* before and *(b)* after milling. No additional material was added. Note the different appearance of the enamel and dentin/cementum components.

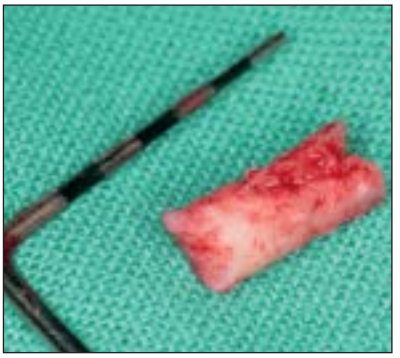


Fig 3 Bone cylinder collected at the time of implantation for histologic/immunohistochemical investigation.

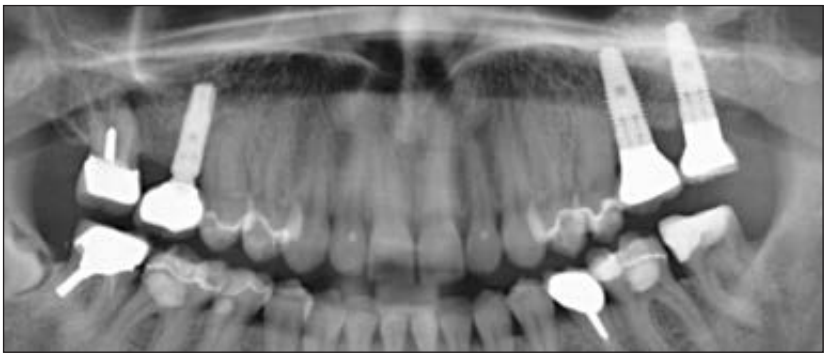
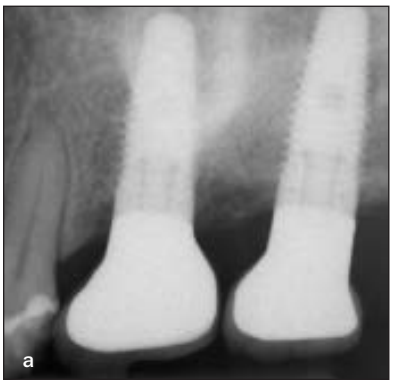


Fig 4 Panoramic reconstruction of digital volume tomography (DVT) 3 months after implant placement on the left side of the maxilla. Note the starry sky-like appearance of the milled tooth material.



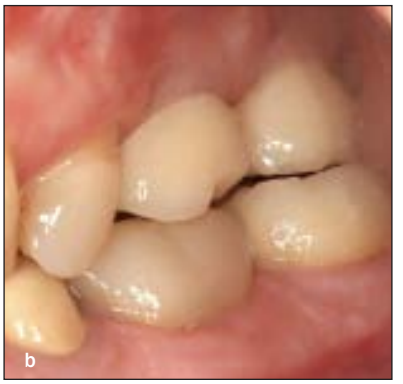


Fig 5 *(a)* Radiograph and *(b)* clinical picture of same patient as in Fig 4 60 months after implant placement.

height were measured in relation to the upper implant edge. Measurements were done after calibration based on the known implant diameter.

Processing of Histologic Specimens

The hard-tissue cylinders of four patients were processed histologically using the cutting-grinding technique; the tissue samples of two other patients were processed for immunohistochemical evaluation. Preparation of the hard thin sections was done according to Donath.[14] For this purpose, the preparations were dehydrated in a graded alcohol series and then embedded in Technovit (Technovit 7200 VCL + BPO, Kulzer & Co). Preparation of the section was done using saws and grinders (Exakt Cutting and Grinding Equipment, Exakt Apparatebau). These were then stained with Levai-Laczko and evaluated histologically. The images were done with a microscope (Olympus BX 51) and a digital camera (XC 10, Program Dotslide).

Processing of the Immunohistochemical Probes

After the bone cylinders were harvested, they were collected in saline and subsequently fixed in 10% formalin for 3 days and then embedded in paraffin after decalcification. After deparaffination through xylene and graded alcohol, the specimens were cut into slices of approximately 4 to 6 µm. For removal of endogenous peroxidase, the material was treated with 3% H_2O_2, followed by antigen retrieval in the microwave (10 minutes, 150 W) in citrate buffer (pH = 6.0) as previously described.[15] Sections of paraffin-embedded bone cylinders were treated with mouse serum and subsequently incubated with mAbs specific for CD 31 (5 µg mouse mAb/mL, DakoCytomation), CD 34 mAb (2.5 µg mouse mAb/mL, Novocasta, Leica Biosystems), α-SMA (1.25 µg mouse mAb/mL, DakoCytomation), type IV (1.25 µg mouse mAb/mL, Ventana Medical Systems, Roche Diagnostics), and vWF (2 µg rabbit mAb/mL, DakoCytomation) at 4°C overnight. Sections were then washed three times in phosphate-buffered saline (PBS), and the reactivity of the primary antibodies was revealed using biotinylated anti-mouse or anti-rabbit IgG (1.25 µg mouse mAb/mL, DakoCytomation; rabbit 10 µg/mL, Vector Biolaboratories) for 30 minutes at room temperature. The unbound secondary antibody was removed by washing three times in PBS, and visualization of antibody staining was achieved using Vectastain ABC (Vector Biolaboratories) and DAB (Santa Cruz Biotechnology). Omission of primary antibody and isotope controls were included in the protocol. Histologic images were taken with a microscope (Leica Microscope, Aristoplan) and an Olympus Camera (Olympus Camera, DP26), with the relevant Imaging Analysis Software system.

Statistical analysis was done in a descriptive manner (IBM SPSS, IBM).

Table 1 Clinical Data of Sinus Elevation Procedure

Patient	Indication	AM	SL / SL + Impl	DT	Histo	I Nr
1	Terminal gap	T/B/ BBM	SL	18	26	2
2	Terminal gap	T/B	SL	28	26	3
3	Terminal gap left, Intermediate gap right	T/B/ BBM	SL + Impl	28		4
4	Single tooth gap	T/B	SL	18	16	2
5	Terminal gap	T/B	SL	28	26	2
6	Terminal gap	T/B	SL	28	26, 27	2

FDI tooth-numbering system.
AM = augmentation material; SL = sinus lift with staged implant placement; SL + Impl = sinus lift together with implant placement; DT = donor tooth; histo = place of histologic specimen collection; INr = number of implants; T = autogenous tooth; B = autogenous scratched bone; BBM = bovine bone mineral.

RESULTS

Clinical Data

Six patients (one male, five female) with an average age of 40 years were treated during the time from October 2008 to September 2013 using the autogenous tooth material. All impacted teeth being used as augmentation material were harvested from the maxilla. In four of the patients, a mixture of bone/tooth material was exclusively used, while the remaining two patients had additional bovine bone substitute material added (Table 1).

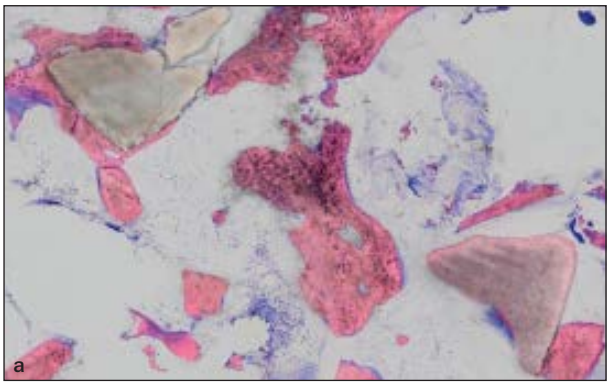
All patients showed uneventful healing, and an overall 15 implants could be placed at the scheduled dates. None of the implants showed any inflammatory processes such as peri-implantitis or peri-mucositis, and bleeding could not be induced by probing in any of the patients. The results of the measurement of the individual and median peri-implant probing depths are shown in Table 2. Pocket depth mostly ranged between 1 and 2 mm and never exceeded a value of 3 mm.

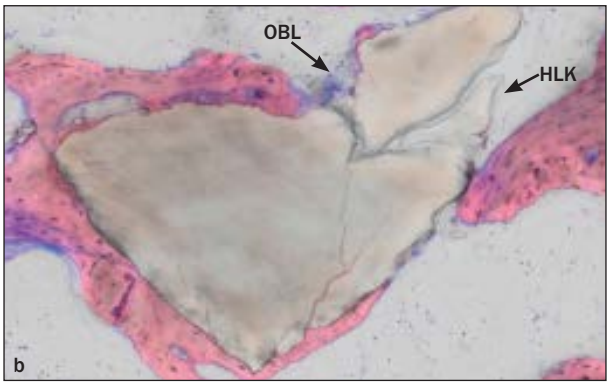
Data for six patients were available for the evaluation of the 2-year results, data for 5 patients for the 3- to 4-year results, and data for two patients for the 5-year results. The peri-implant bone resorption during the first year was an average 0.3 mm, with a minimum of 0 mm and a maximum of 1.8 mm. Peri-implant bone resorption was found to be virtually stable for the remaining follow-up period following an initial bone loss during the first year of function (Table 2).

Table 2 Clinical Data of Peri-implant Measurements

		Time point (mo)							
		0		3		6		12	
Patient	I loc	pd	br	pd	br	pd	br	pd	br
1	15	0	0	1.75	0	1.7	0.22	1.75	0.73
	16	0	0	2.5	0	2.6	0	2.75	0
2	16	0	0	1.75	0	2	0	2	0
	26	0	0	1.25	0.35	1.2	0.48	1.25	0.88
3	27	0	0	1	0.25	0.9	0.5	1	0.83
	25	0	0	1.25	0.15	1.2	0.7	1.25	0.95
	27	0	0	1.25	0.2	1.1	0.8	1.25	0.9
	14	0	0	1.75	0.45	1.9	1.5	2	1.68
	15	0	0	1.75	0	1.7	0	2	0
4	16	0	0	2	0	2	0	2.25	0
	26	0	0	1.5	0	2	0	2	0
5	25	0	0	1.75	0	2.25	0.35	2.25	0.85
	26	0	0	1.5	0	1.6	0.3	1.75	0.7
6	26	0	0	2.25	0	2	0	2.25	0
	27	0	0	2	0	2.1	0	2.25	0
Mean ± SD	**0**	**0**	**0**	**1.68 ± 0.41**	**0.09 ± 0.15**	**1.75 ± 0.47**	**0.32 ± 0.43**	**1.87 ± 0.49**	**0.5 ± 0.53**

FDI too-numbering system
I loc = implant location; pd = average peri-implant probing depth at the individual time point; br = average perii-implant bone resorption at the individual time point; SD = standard deviation.





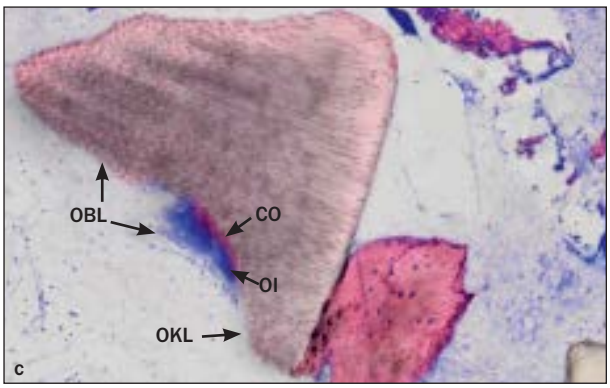


Fig 6a Ground section showing enamel and dentin particles partially covered by woven and lamellar bone (Levai-Laczko).

Fig 6b Detail from Fig 6a. Note the irregular surface of enamel corresponding to Howship's lacunae (HLK). Osteoblasts (OBL) on uncovered surface of the enamel.

Fig 6c Detail from Fig 6a. Note the different stages of bone building: OKL = osteoclast; OBL = osteoblast; OI = osteoid; CO = calcified osteoid; woven bone with osteocytes *(blue)*.

Time point (mo)							
24		36		48		60	
pd	br	pd	br	pd	br	pd	br
1.75	0.73	2	0.75	2.15	0.93	2.5	0.73
2.75	0	2.75	0	2.7	0	3	0
1.75	0	1.75	0	1.75	0	1.75	0
1.25	0.91	1.3	0.92	1.35	1.11	1.75	1
1	0.83	1	0.81	1	0.1	1	0.93
1.25	1.07	1.5	1	1.65	1.14		
1.75	0.9	2	0.8	2.15	0.98		
2.5	1.68	2.75	1.7	2.6	1.77		
1.75	0	1.75	0	1.7	0		
2.25	0	2.3	0	2.35	0		
2	0	2	0	2	0		
2.25	0.85	2.3	0.81	2	1.06		
2	0.89	2	0.85	1.8	1.27		
3	0						
2.25	0						
1.97 ± 0.56	0.52 ± 0.55	1.95 ± 0.55	0.59 ± 0.54	1.94 ± 0.47	0.64 ± 0.64	2 ± 0.77	0.53 ± 0.5

Results of Histologic Examination

Overall, six biopsy specimens from five patients were available for histologic/immunohistochemical evaluation (Figs 6a to 6c and 7). The average in situ time of the augmentation material after sinus elevation grafting was 6.6 months (minimum 4 months, maximum 10 months). All biopsy specimens were collected after two-step sinus elevation grafting in the course of implant placement. One additional material could be harvested 15 months after augmentation due to an exostosis-like formation approximately 0.5 cm mesial of the former sinus-elevation window on the buccal aspect.

The core biopsy specimens contained varying degrees of dentin and enamel showing osteoclastic resorptions (Howship's lacunae) with giant cell coating on the surface. As a result, the autogenous material was colonized by osteoblasts and covered with newly formed bone in a wallpaper-type manner, and the individual particles were fused by widened bone bridges. Osteogenesis appeared to originate from the local bone bed. There was no compulsive evidence of orthotopic new bone formation in the form of osteoinduction and, consequently, no evidence of any primary conversion of the tooth material to autogenous bone. On the whole, the enamel material appeared to be more extensively coated with new bone than the dentin. Shares of the cementum could not be identified with absolute certainty.

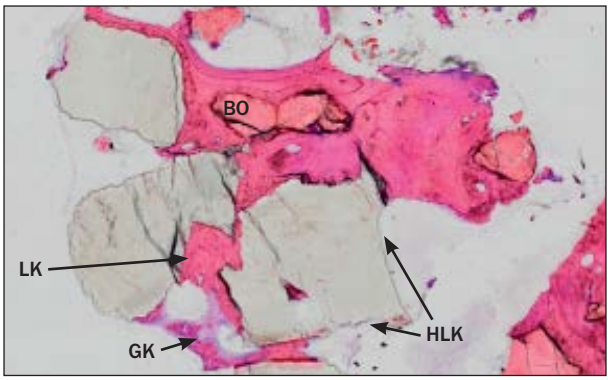


Fig 7 Enamel particles surrounded by bone in different stages of maturity: GK = woven bone; LK = lamellar bone; BO = bovine bone mineral; HLK = Howship's lacunae.

In cases in which additional bovine substitute material had been used, osteoconductive bone neogenesis could be observed as already described previously.[16]

Results of Immunohistochemistry

Drill cylinders containing the reorganized reconstitution tooth material used for pre-implantologic sinus

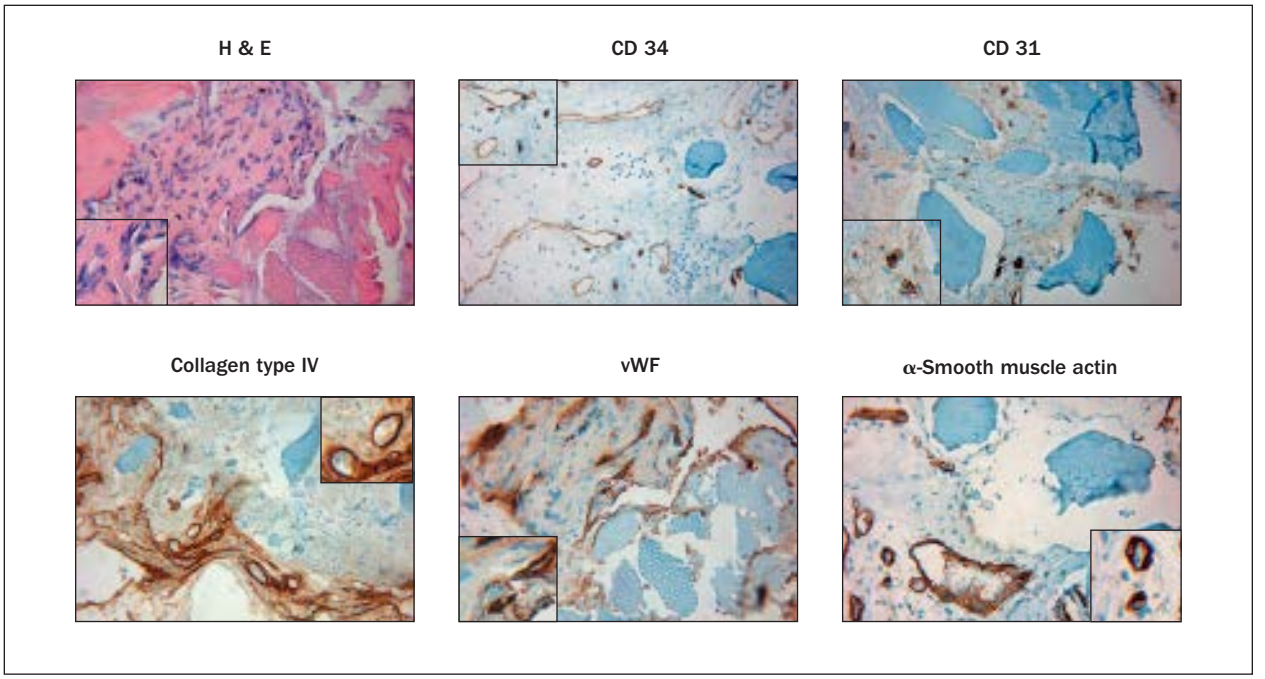


Fig 8 Drill cylinders containing the remodeled reconstitution tooth material were stained with hematoxylin-eosin (H & E) or Abs specific for CD 34, CD 31, collagen type IV, vWF, or α-smooth muscle actin according to material and methods and counterstained with Mayer's hemalum. The primary antibody was omitted for control purposes, and isotype controls were included in the protocol and showed no staining. (Original magnification ×150, in inserts ×400).

maxillary grafting showed segmental bone tissue remodeling with intensely eosinophilic bone matrix mixed with dentin fragments degraded by osteoclasts inducing resorption lacunae. Vessel distribution and density could be analyzed using immunohistochemical staining with endothelial cell markers CD 31, CD 34, vWF, and a marker for basal membrane collagen type IV as well as for smooth muscle actin (Fig 8). Interestingly, the interposed stroma was loosely organized and highly vascularized by smaller and larger capillaries. Intense new vessel formation could be observed in the area of reorganized tissue of the augmented area, which was primarily avascular. This effect was observed without the addition of vasculoinductive mediators from platelets or single provascular factors. Almost no inflammation was observed as indicated by leukocyte infiltration and occurrence of lymphocytes in perivascular areas.

DISCUSSION

The investigation presented reports on a new method for sinus elevation grafting using autogenous, chemically unaltered tooth material as bone substitute material in a consecutive cohort of six patients. To avoid potential contamination, only patients with fully impacted third molars were eligible for inclusion. In one patient, a connection of the crown section with the oral cavity could not be definitely excluded, so only the root section without the crown of this tooth was used. All the remaining patients received all portions of the removed third molar.

The results of this investigation comprised a period of up to 5 years. No signs of maxillary sinusitis or of other clinical complications such as implant loss, peri-mucositis, or peri-implantitis could be observed, and all implants were prosthetically treated within the scheduled time frame.

Peri-implant pocket depth as well as the results of bleeding upon probing were consistent with the criteria for successful implants.[17] The results obtained were also consistent with those for implants that had been inserted into local, nonaugmented bone,[18] or those with a preceding sinus elevation with different augmentation materials.[19]

Peri-implant bone resorption in the present study was an average 0.3 mm, and none of the values measured exceeded 2 mm. Thus, all implants also fulfilled the most common, but only slightly varying radiographic success criteria.[17,20–23]

No implant was lost, but the number of implants placed was relatively small, and the follow-up period was still limited. However, as neither clinical nor radiographic findings suggested the presence of peri-implantitis or peri-mucositis, a good long-term prognosis can be assumed and anticipated for these implants.

Processing of the histologic specimens obtained from all of the patients with two-stage implant insertion (five

out of six patients) allowed assessment of the integration of the tooth material and the response of the local bone site. Osteoclasts that originate from local tissue attach to dentin and enamel, partly resorbing these tissues at the surface. Further, the grafted autogenous hard tissue was covered by osteoblasts depositing osteoid being calcified in a second step. The woven bone thus generated was then increasingly converted into lamellar bone. The encapsulated tooth material remained partly immured. While no evidence of remodeling after bony encapsulation was found in the specimens, such remodeling is very well feasible and should even be anticipated by analogy with the processes following dental trauma. Such processes may possibly be verified in animal experimental studies and by processing of full-tissue blocks. The osteoconductive characteristics were consistent with those described for commercial preparations in vivo[19] and in vitro.[24]

These findings are in contrast to those reported by various other authors[25,26] who did not observe any osteoconductive properties of dentin or other dental components. The reasons for these diverging findings remain unclear and will need to be studied in additional investigations. It may be imaginable that animals dispose of a mechanism of osteogenesis associated with autogenous tooth material being different to that of humans. In any case, osteoconductivity of dental components could also be observed in an animal model in rats when using decalcified tooth material mixed with HPC,[10] so decalcified dentin has been used experimentally as a carrier material for bone morphogenetic protein (BMP).[27]

Obviously, the changes observed histologically in the augmentation material in the present study are similar and comparable to those seen after extrusive or intrusive dental trauma; upon significant pulpal and periodontal damage, vascular neogenesis with resulting resorption of the root surface will develop. This will result in new formation of cementum or—in the case of a direct connection between bone and root surface—in root resorption being consecutively replaced by new bone formation.[28]

One of the advantages of autogenous tooth material for the suggested use involves the fact that neither deproteinization nor any other additional chemical or physical treatment is required, which is the case for other commercially available substitute materials.[29,30] Autogenous tooth material as grafting material is not associated with any risk of allopathic and xenopathic transmission, and its powder consistency may favor a flexible use with other grafting materials such as autogenous platelet-rich fibrin, platelet-rich plasma, or thrombin-activated platelet supernatant that favor optimal bone regeneration.

Strikingly, no cementum could be demonstrated in any of the specimens. The tooth itself consists of a roughly equal volume share of cementum and tooth enamel, so an appropriate cementum share also had to be expected in the histologic biopsy specimens. The reasons why no cementum fragments could be observed in the histologic specimens can only be explained hypothetically: It is likely that extensive incorporation had taken place, no longer allowing for any histologic differentiation at the time of examination on account of the close resemblance of the cementum to bone. Additional studies, partly with shorter observation periods, will be needed to identify the fate of cementum in the autogenous implant bed.

In addition to pulpal stem cells and undifferentiated neural crest cells, tooth material also contains signal molecules such as osteopontin[10] and in a decalcified state may transform mesenchymal stem cells into osteoblasts,[31] making osteogenesis by osteoinduction principally conceivable. It has been suggested that this effect is the result of the release of BMP from dentin and a surface enlargement caused by the exposure of dentinal tubules and collagenous fibers.[11] This type of osteogenesis would thus be observed in an isolated location and not in the vicinity of local bone. While isolated locations principally allowing for such a conclusion were identified in the present study, only the full processing of complete specimens will provide detailed information. Thus, any assessment based on histologic biopsy specimens covering only a part of the augmented section will be impossible.

In the present study, shares of the patients' own bone collected using a bone scraper had been added to the milled tooth material. This involved a simple procedure for collecting particulate cortical bone (length, 0.9 to 1.7 mm; thickness, 100 µm). Histologic differentiation of these autogenous bone grafts was no longer possible in any of the ground sections. Histologically, the bovine bone mineral that had been added in two of the six patients did not show any other behavior when used alone and had already been described earlier,[16] and the tooth material did not change bone-building properties. In cases of insufficient tooth material for augmentation, adding bovine bone mineral seems to be feasible.

Selective vascular staining could show an intense new vessel formation observed in the area of reorganized tissue of the augmented area. Histomorphometric evaluation did not appear meaningful on account of the small number of samples. Considering the excessive new vessel formation, continued and extensive formation of new bone can be assumed since a close spatial relationship between angiogenesis and osteogenesis could be demonstrated by higher microvascular density in areas that were subject to new bone formation and areas where maturation processes were occurring in formerly elevated sinuses.[15]

CONCLUSIONS

Based on the results of the present preliminary case report and considering the limited number of cases, the use of autogenous nondecalcified tooth material for augmentation in sinus elevation grafting may be recommended. Additional, especially experimental, studies should be performed to clarify the exact mechanism, in particular with regard to a potential osteoinductive component of new bone formation.

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