

Does an autogenous demineralized dentin (ADDM) graft has the ability to form a new bone?

ABSTRACT

Aim: This aimed to evaluate a new bone formation and to provide a single-stage treatment, i.e., extraction of tooth followed by autogenous dentin demineralized (ADDM) graft in the same extraction socket (ES) – for adult patients who require tooth extraction.

Materials and Methods: Two hundred teeth extractions were performed to investigate the efficacy of ADDM graft in the formation of new bone. After demineralization of dentin graft parameters such as exposure of graft, any signs of infections/pus/exudates, pain, and bone density were evaluated.

Results: On follow-up, exposure of graft was recorded in five sockets at 1 month and infection was recorded in four sockets. Pain was significantly high at postoperative day 1 and least with 3rd and 6th months. There was a highly significant bone formation ($P < 0.01, 0.05$) in the ES group at various time intervals, while there were no significant differences in the adjacent bone group.

Conclusion: Using ADDM graft in providing a single-stage treatment plan, i.e., extraction of tooth followed by autogenous demineralized dentin graft in the same ES in adult patients – is an alternative for the immediate reconstruction of alveolar bone defects to facilitate the future prosthesis. It also saves the cost of other graft materials which are commercially available in market for the patient and also reduces the infective dental waste globally.

Keywords: Autogenous demineralized dentin graft (ADDM), autogenous graft, bone density, dentin graft

INTRODUCTION

Extracted human teeth are considered as an infective waste globally and their sockets are generally left untreated for physiologic healing.^[1] Reduction of 2.6–4.6 mm in width and 0.4–3.9 mm in height of alveolar socket was reported during the healing phase.^[2] The bone volume reduction following teeth extractions by 50% within 12 months and 2/3 of this resorption in the first 3 months have been reported by some studies.^[3,4] Due to the bone resorption, there is a loss of socket width three dimensionally resulting in narrowing and shortening of the alveolar ridge contour.^[5,6] Therefore three-dimensional bone volume maintenance is obligatory for ideal esthetic and functional outcomes.

Bone is the second most transplanted tissue after blood.^[7] Ideal bone graft material requires osteogenesis, osteoinduction, and osteoconduction, in combination or alone for bony

healing.^[8] The selection of an ideal bone graft relies on several factors such as tissue viability, defect size, graft size, shape and volume, biomechanical characteristics, graft handling, cost, ethical issues, biological characteristics, and associated complications.^[9] The materials used in bone grafting can be divided into several major categories, including autografts,

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allografts, xenografts, and synthetic bone graft substitutes. Merits and demerits of these grafts have been mentioned in literature and autografts are the “gold standard” for reconstructing the bony defects.^[10,11]

The present study was conducted with aimed to provide a single-stage treatment plan – extraction of tooth followed by autogenous demineralized dentin graft in the same extraction socket (ES) – for adult patients who require tooth extraction.

MATERIALS AND METHODS

The present single-group, prospective, randomized, clinical study was conducted after institutional ethical clearance with a total of 200 unilateral/bilateral, maxillary/mandibular, single/multiple posterior teeth being treated in August 2016 and November 2019. The study was carried out to investigate the efficacy of autogenous dentin demineralized (ADDM) graft in the formation of new bone, and the treatment outcome of ADDM graft in patients who belonged to age group above 18 years with an inclusion criterion of the teeth which were not treated with root canal therapy. Exclusion criteria were below 18 years of age, patients with significant medical comorbidities, for example, uncontrolled diabetes, bleeding disorder, autoimmune disorder, history of malignancy, radiation, pregnancy, use of long-term steroid therapy/antibiotics, radiological abnormalities like the presence of cyst/tumor, smoker, and patients who not willing to give their consent.

Collection of data

- A. Preoperative clinical and radiological evaluation: Detailed history and complete clinical examination, routine hemogram, and radiological examination by orthopantomograph (OPG) were performed. The proposed surgical procedure with its merits and demerits was explained to the patient and written consent was obtained.
- B. After a gargle with 0.1% chlorhexidine solution, patients were injected with 2% lignocaine with 1:100,000 adrenaline. During extraction, extreme care was taken to preserve the surrounding bony and soft tissues. After cleaned tooth extraction, sockets were debrided and rinsed with saline. All carious lesions, restorations such as crowns and fillings, calculus if any, were removed. Layers of enamel, discolored dentin, and cementum were also removed. The pulp tissue from the root canal(s) was removed using root canal instruments. The teeth were washed with sterile normal saline. Subsequently, tooth dentin was grinded with “mortar and pestle” to prepare 0.25–2 mm particles,

which was confirmed using the sieving method. For demineralization, these particles were immersed in basic alcohol (0.5M of NaOH and 30% alcohol [v/v]) for 10 min in a sterile container for defatting, dissolving all organic debris, bacteria, and toxins of the dentin particulate. After decanting the basic alcohol cleanser, the particulate was washed thrice in phosphate buffered saline (PBS). The PBS was decanted leaving wet particulate dentin ready to graft into freshly extracted sockets. The graft was carefully inserted into the alveolar socket followed by suturing

- C. Evaluation of clinical and radiographic parameters:
 - a. Exposure of graft and any signs of infections/pus/exudates were measured at 1, 3, and 6 months postoperatively by only observation
 - b. Pain was measured immediately before the surgery and on follow-up after postoperative days 1, 3, and 7, and months 1, 3, and 6 using the Numeric Pain Rating Scale. The 11-point numeric scale ranged from “0”-no pain to “10”-worst pain
 - c. Bone density measurement was performed with OPGs. To compare and evaluate bone densities in ES (Group 1) and adjacent bone (AB) (Group 2), OPGs were taken at preoperatively, immediate after extraction (IAE), immediate after grafting (IAG), and at 1 (A1), 3 (A3), and 6 (A6)-month follow-up. The photos, digitalized as Digital Imaging and Communications in Medicine files, were converted into JPEG graphic files and the JPEGs were stored. From the stored files, bone densities were measured in the area. The average value of the tonality gray scale was used as the gray-level histogram of the Adobe Photoshop CS2 program. The gray values were related to the absorption of X-rays, the radiologic density of a certain tissue. The gray values were saved in an 8-bit color space. Every pixel obtained a value from 0 to 255 in which 0 stands for black, low radiologic density, while 255 for white, total X-ray absorption.^[11]

Statistical procedures

Data obtained were compiled on an MS Office Excel Sheet (version 2010, Microsoft Redmond Campus, Redmond, Washington, USA). Data were subjected to statistical analysis using Statistical Package for the Social Sciences (SPSS) (SPSS version 21.0, IBM, New York, USA). Descriptive statistics such as frequencies and percentages for categorical data and mean and standard deviation for numerical data have been depicted. Normality of numerical data was checked using Shapiro–Wilk test and was found that the data followed a normal curve in the measurement of pain; hence, intragroup comparison was performed using ANOVA (for >2

observations) followed by *post hoc* test. While in measuring bone density, the data did not follow a normal curve; hence, intergroup comparison (two groups) was done using Mann–Whitney *U*-test and intragroup comparison using Friedmans (for >2 observations) followed by Wilcoxon signed-rank test. For all the statistical tests, $P < 0.05$ was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving a power to the study as 80%.

* = significant difference ($P < 0.05$)

** = highly significant difference ($P < 0.01$)

= nonsignificant difference ($P > 0.05$).

RESULTS

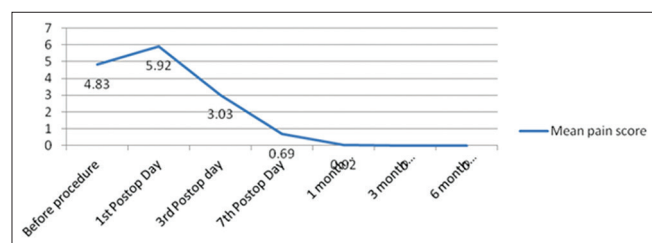
Eight-eight females and 122 males with periodontal problems (37.5%) remained the most common etiologic factor for teeth extraction followed by prosthodontic rehabilitation (25.5%), endodontic (19.5%), orthodontic considerations (8%), prophylactic removal (5%), and miscellaneous (4.5%) like nonaffordability and far distance.

Exposure of graft was recorded in five ESs at 1 month follow-up. Infection was recorded in four ESs.

When comparisons of pain were performed in the ES group, there was a statistically highly significant difference seen for the values between the time intervals ($P < 0.01, 0.05$) with higher values at 1-day postoperative and least values at A3 and A6 postoperative [Graph 1].

Pairwise comparison using Scheffe *post hoc* test was represented in time 1–7: 1 – before procedure, 2 – 1st postoperative day, 3 – 3rd postoperative day, 4 – 7th postoperative day, 5 – 1st postoperative month, 6 – 3rd postoperative month, and 7 – 6th postoperative month. There was a statistically significant difference seen for the values between all the pairs of time intervals ($P < 0.01, 0.05$) except for 5 versus 6, 5 versus 7, and 6 versus 7 where there was a statistically nonsignificant difference seen for the values between the time intervals ($P > 0.05$) [Table 1].

There was a statistically highly significant difference seen for the values between the groups ($P < 0.01, 0.05$). For IAE with



Graph 1: Comparison of mean pain score at different time intervals

higher values in group AB, IAG with higher values in group ES, A1 with higher values in group ES, A3 with higher values in group ES, and A6 with higher values in group ES [Table 2].

DISCUSSION

The study recruited a total of 224 ESs, although initially it was decided to include 200, since the study was a long-term follow-up of 6 months, it was decided to over recruit subjects to avoid loss of samples by attrition. At the end of the study, 24 were lost to follow-up which encountered to 12% attrition rate.

In 1967, Bang and Urist confirmed the bone-inducing property of dentin in 520 sample size of a rabbit.^[12] Urist verified that completely demineralized dentin matrix (DDM) induced bone at 4 weeks, while nondemineralized dentin induced at 8–12 weeks. Therefore, DDM had a more active bone-inducing matrix than the calcified dentin.^[13]

Very interestingly, Reddi in 1974 mentioned that the demineralized treatment for bone and dentin increases their osteoinductivity and decreased their antigenicity.^[14] Finkelman *et al.* concluded that dentin and bone are mineralized tissues and almost have a similar chemical component. Both DDM and DBM are composed of predominantly Type I collagen (95%) and the remaining as noncollagenous proteins including a small amount of growth factors.^[15] In the regenerative field, biomaterials must allow both bone formation and gradually absorb as to be replaced by bone. Nonabsorbable materials are never replaced by bone and thus reveal chronic inflammation in tissues as foreign bodies. Bone-inductive, absorbable properties of dentin have been studying a medical recycle of human teeth as a novel graft material for bone regeneration in Japan and Korea.^[16]

Mônica Fernandes compared the bone repair in the experimental group (ADDM) with the control group (no ADDM). After demineralization with 0.6 N hydrochloric acid, the ADDM stimulated faster new bone formation and was completely resorbed during the bone remodeling than in the control group.^[17] Masaru Murata had crashed the tooth in a 0.4–0.8 mm size with liquid nitrogen and demineralized in HCl. They found that DDM was osteoinductive matrices on 32-week follow-up, and therefore, DDM can be effective as a carrier of BMP-2 for bone engineering.^[18]

Sangeetha *et al.* compared routine decalcification methods with microwave decalcification of bone and teeth using the solutions of nitric acid (5%), formic acid (5%), and EDTA (14%) and concluded that the microwave method using nitric

Table 1: Pairwise comparison using Scheffe post hoc tests

For this table time 1, 2, 3, 4, 5, 6, 7 is as follows:
 1. Before procedure
 2. 1st postoperative day
 3. 3rd postoperative day
 4. 7th postoperative day
 5. 1 month postoperative
 6. 3 months postoperative
 7. 6 months postoperative

Time (I)	Time (J)	Mean difference (I-J)	SE	P	95% CI	
					Lower bound	Upper bound
1	2	-1.095*	0.156	0.000**	-1.65	-0.54
1	3	1.795*	0.156	0.000**	1.24	2.35
1	4	4.135*	0.156	0.000**	3.58	4.69
1	5	4.810*	0.156	0.000**	4.25	5.37
1	6	4.825*	0.156	0.000**	4.27	5.38
1	7	4.825*	0.156	0.000**	4.27	5.38
2	3	2.890*	0.156	0.000**	2.33	3.45
2	4	5.230*	0.156	0.000**	4.67	5.79
2	5	5.905*	0.156	0.000**	5.35	6.46
2	6	5.920*	0.156	0.000**	5.36	6.48
2	7	5.920*	0.156	0.000**	5.36	6.48
3	4	2.340*	0.156	0.000**	1.78	2.90
3	5	3.015*	0.156	0.000**	2.46	3.57
3	6	3.030*	0.156	0.000**	2.47	3.59
3	7	3.030*	0.156	0.000**	2.47	3.59
4	5	0.675*	0.156	0.005**	0.12	1.23
4	6	0.690*	0.156	0.004**	0.13	1.25
4	7	0.690*	0.156	0.004**	0.13	1.25
5	6	0.015	0.156	1.000 [#]	-0.54	0.57
5	7	0.015	0.156	1.000 [#]	-0.54	0.57
6	7	0.000	0.156	1.000 [#]	-0.56	0.56

*Statistically significant difference ($P < 0.05$), **Statistically highly significant difference ($P < 0.01$), [#]Nonsignificant difference ($P > 0.05$). CI: Confidence interval, SE: Standard error

acid was the fastest among all.^[19] Tanuja Penmatsa used a combination of tetracycline with hydrochloric acid for decalcification and concluded that combination has a beneficial effect of anti-inflammatory, enhanced regeneration, and attachment of fibroblasts, anticollagenase activity, and high substantivity.^[20]

Murata reported a first clinical case in 2003 using ADDM graft in sinus lifting and found excellent bone formation on follow-up.^[21] Arafat Kabir used a conventional hand-operated method with stainless-steel vessel and a bar instead of a smart dentin grinder/automill. Crushed tooth was decalcified in 2% HNO₃ for 30 min. They concluded that AutoDDM granules have osteoinductive properties, and therefore, dentin is an alternative material to the bone graft.^[22] Binderman presented a novel procedure to process the extracted teeth for immediate grafting of autogenous dentin with basic alcohol followed by phosphoric-buffered saline (PBS) wash.^[23] In our study, the hand operated vessel and bar was used similar to Arafat Kabir and decalcification method was carried out as Binderman..

Kim *et al.* published a case series with different tooth forms: tooth block, chips, and powder form for socket preservation and dental implants. OPGs presented a good bony healing, while cone-beam computed tomography presented a maintenance of ridge height and width on follow-up.^[24] Joshi *et al.* conducted a prospective, randomized controlled pilot clinical trial of autogenous whole tooth autograft (WTA), dentin allograft (DA), freeze-dried bone allograft (FDBA), and left ungrafted (control) in ESs. They concluded that WTA and DA consistently showed superior results in more new bone formation than FDBA and control groups.^[25]

There was a statistically significant difference in measuring pain for the values in intragroup between the time intervals ($P < 0.01, 0.05$) when measured on ANOVA with highest values at 1-day postoperative day (5.92 ± 2.229) followed by before procedure (4.83 ± 2.467), 3rd postoperative day (3.03 ± 2.169), 7th postoperative day (0.69 ± 1.1666), and 1 month postoperative (0.02 ± 0.1222) and least values at 3 month and 6 month postoperative (0.00 ± 0.000). Although the nature of pain was not compared in preoperative and postoperative phase. The results of the study possibly

will suggest that preoperative pain may be due to the etiology itself such as carious tooth and infection. However, postoperative pain may be allied with inflammatory reaction to postextraction procedure which also disappeared in almost a week of postgraft. In addition, analgesics were given for 5 days in all patients in a postoperative period which cannot be elapsed.

When bone density was compared in ES group, there was a statistically highly significant difference in ES seen for the values between the time intervals ($P < 0.01, 0.05$)

with higher values at A6 (median 172.82000) and least at IAE (mean 74.60000). There was an also statistically highly significant difference in ES seen for the values ($P < 0.01, 0.05$) for all the time pairs, while there was a statistically no significant difference seen for the values ($P > 0.05$) for the following time pairs in AB group at IAG versus A1, IAG versus A6, A1 versus A6, and A3 versus A6 [Tables 3 and 4].

OPGs just immediately after placement of graft revealed radiopaque particles covering fully inside the socket including a sharp radiopaque line of lamina dura. At 1,

Table 2: Bone density: Intergroup comparison extraction socket versus adjacent bone

Group	n	Mean	SD	SEM	Median	Mann-Whitney U-value	Z	P value of Mann-Whitney U-test
IAE								
ES	200	68.161900	33.5884116	2.3750594	74.6	9330.000	-9.232	0.000**
AB	200	104.831100	25.6039393	1.8104719	98.85			
IAG								
ES	200	157.428200	20.7078775	1.4642681	157.77	2643.000	-15.017	0.000**
AB	200	107.113700	26.5671408	1.8785805	105.82			
A1								
ES	200	160.057600	29.7568888	2.1041298	164.95	3419.000	-14.348	0.000**
AB	200	106.563750	23.7520048	1.6795204	99.87			
A3								
ES	200	166.159500	26.4783799	1.8723042	168.87	2488.000	-15.151	0.000**
AB	200	108.444000	20.6533636	1.4604133	106.16			
A6								
ES	200	169.820500	25.9920622	1.8379163	172.82	2035.000	-15.545	0.000**
AB	200	105.421150	28.5890424	2.0215506	107.32			

**Statistically highly significant difference ($P < 0.01$). ES: Extraction socket, AB: Adjacent bone, IAE: Immediate after extraction, IAG: Immediate after graft, A1: After 1 month postoperative, A3: After 3-month postoperative, A6: After 6-month postoperative, SD: Standard deviation, SEM: Standard error of mean

Table 3: Bone density: Pairwise comparison for extraction socket (n=200)

	Mean	SD	SEM	Z	P value of Wilcoxon Signed rank test
IAE ES	68.161900	33.5884116	2.3750594	-12.263	0.000**
IAG ES	157.428200	20.7078775	1.4642681		
IAE ES	68.161900	33.5884116	2.3750594	-12.259	0.000**
A1 ES	160.057600	29.7568888	2.1041298		
IAE ES	68.161900	33.5884116	2.3750594	-12.260	0.000**
A3 ES	166.159500	26.4783799	1.8723042		
IAE ES	68.161900	33.5884116	2.3750594	-12.262	0.000**
A6 ES	169.820500	25.9920622	1.8379163		
IAG ES	157.428200	20.7078775	1.4642681	-7.669	0.000**
A1 ES	160.057600	29.7568888	2.1041298		
IAG ES	157.428200	20.7078775	1.4642681	-7.884	0.000**
A3 ES	166.159500	26.4783799	1.8723042		
IAG ES	157.428200	20.7078775	1.4642681	-8.397	0.000**
A6 ES	169.820500	25.9920622	1.8379163		
A1 ES	160.057600	29.7568888	2.1041298	-9.170	0.000**
A3 ES	166.159500	26.4783799	1.8723042		
A1 ES	160.057600	29.7568888	2.1041298	-10.073	0.000**
A6 ES	169.820500	25.9920622	1.8379163		
A3 ES	166.159500	26.4783799	1.8723042	-7.708	0.000**
A6 ES	169.820500	25.9920622	1.8379163		

**Statistically highly significant difference ($P < 0.01$). ES: Extraction socket, IAE: Immediate after extraction, IAG: Immediate after graft, A1: After 1-month postoperative, A3: After 3-month postoperative, A6: After 6-month postoperative, SD: Standard deviation, SEM: Standard error of mean

Table 4: Bone density: Pairwise comparison for adjacent bone (n=200)

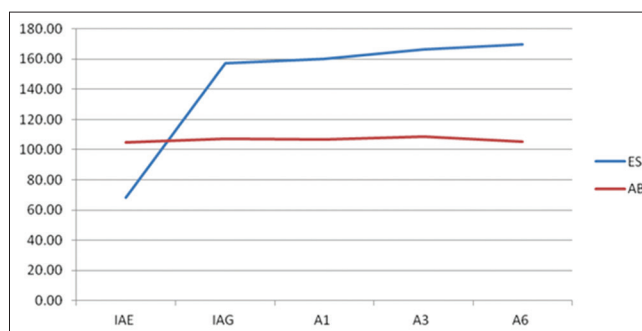
	Mean	SD	SEM	Z	P value of Wilcoxon Signed rank test
IAE AB	104.831100	25.6039393	1.8104719	-4.018	0.000**
IAG AB	107.113700	26.5671408	1.8785805		
IAE AB	104.831100	25.6039393	1.8104719	-2.317	0.021*
A1 AB	106.563750	23.7520048	1.6795204		
IAE AB	104.831100	25.6039393	1.8104719	-6.450	0.000**
A3 AB	108.444000	20.6533636	1.4604133		
IAE AB	104.831100	25.6039393	1.8104719	-2.474	0.013*
A6 AB	105.421150	28.5890424	2.0215506		
IAG AB	107.113700	26.5671408	1.8785805	-0.690	0.490#
A1 AB	106.563750	23.7520048	1.6795204		
IAG AB	107.113700	26.5671408	1.8785805	-6.542	0.000**
A3 AB	108.444000	20.6533636	1.4604133		
IAG AB	107.113700	26.5671408	1.8785805	-1.422	0.155#
A6 AB	105.421150	28.5890424	2.0215506		
A1 AB	106.563750	23.7520048	1.6795204	-8.078	0.000**
A3 AB	108.444000	20.6533636	1.4604133		
A1 AB	106.563750	23.7520048	1.6795204	-0.828	0.408#
A6 AB	105.421150	28.5890424	2.0215506		
A3 AB	108.444000	20.6533636	1.4604133	-0.055	0.956#
A6 AB	105.421150	28.5890424	2.0215506		

*Statistically significant difference (P<0.05), **Statistically highly significant difference (P<0.01), #Nonsignificant difference (P>0.05). AB: Adjacent bone, IAE: Immediate after extraction, IAG: Immediate after graft, A1: After 1-month postoperative, A3: After 3-month postoperative, A6: After 6-month postoperative, SD: Standard deviation, SEM: Standard error of mean

3, and 6 months of the follow-up, the alveolar socket appeared to be filled with uniform radiodense bone-like tissue indicated that the socket healed fully with new bone. In addition, the lamina dura of the socket disappeared completely by bone remodeling. Gradual absorption of the demineralized granules was observed on the sequential radiographic findings of the socket. Furthermore, the results of intergroup comparison were done, and it revealed that after the placement of DDM graft, there was a significant bone formation which can also be appreciated on the linear graph, while there were no significant differences in AB in time frames of immediately after grafting of AB with 1-month and 6-month follow-up [Graph 2]. OPGs were used in this study to avoid high-radiation doses and high costs.

Exposure of graft was recorded in five ESs at 1-month follow-up. Infection was recorded in four ESs and they were treated with the removal of graft followed by deep curettage and antibiotics. After a close follow-up of the said subjects, the wound healing was satisfactory.

Leaving the study outside the scope because of the larger sample size, the following ideas could be tested: first, it could be interesting to consider a histological evaluation of newly formed tissues. Second, measurements of the absolute mineral density could be added to estimate the exact amount of bone formation. Third, further studies should be performed to confirm the biological safety of this



Graph 2: Comparison of bone density in ES and AB group. ES: Extraction socket, AB: Adjacent bone, IAE: Immediate after extraction, IAG: Immediate after graft, A1: After 1-month postoperative, A3: After 3-month postoperative, A6: After 6-month postoperative

tooth-based graft material. Another field of further research is a measurement of above-mentioned parameters using the DDM grafts in the techniques of direct and indirect sinus lift, nasal floor elevation, etc., in implant dentistry. More studies are required to establish a histological and biological safety of ADDM graft.

CONCLUSION

This study is a large case series using ADDM graft in providing a single-stage treatment plan, i.e., extraction of tooth followed by autogenous demineralized dentin graft in the same ES in adult patients – is an alternative for the immediate reconstruction of alveolar bone defects to facilitate the future prosthesis. It also saves the cost of other graft materials which

are commercially available in market for the patient and also reduces the infective dental waste globally.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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