DFDBA grafting versus natural healing after extraction: A Randomised Controlled Clinical Trial

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Abstract

The aim of this study was to determine the quality of bone available for implant placement using DFDBA as grafting material in combination with a resorbable collagen membrane, compared to bone in extraction sockets that were left to heal naturally. A total of 20 sites were identified from eight patients requiring replacement of two or more extracted teeth by means of dental implant supported structures, on contralateral sides of the same jaw. They received DFDBA grafting of the socket on one side and no grafting on the contralateral side at the time of extraction. When implants were placed 16 – 20 weeks later, core samples of bone from these sites were first harvested by means of a trephine drill and those samples were processed and examined histologically to determine which of these sites displayed better quality of bone. One patient's samples could not be utilised. Comparing the samples of the remaining nine non-grafted to nine grafted extraction sites, the difference in the calculated percentages of trabecular bone and collagen as well as the numbers of osteocytes, inflammatory cells and blood vessels were statistically insignificant. The results of the study indicate that statistically there are no significant histological differences between DFDBA-grafted and non-grafted sockets.

Introduction

Dental sockets decrease in volume after tooth extraction and change morphologically.[1,2] With dental implant treatment becoming so widespread, the need to preserve bone after tooth extraction has become an ever-increasing concern for clinicians.[3] Recent advances in bone grafting materials and techniques allow dentists to place implants in sites that were considered compromised in the past. It is well documented that post-extraction maintenance of the alveolar ridge volume by grafting the socket may minimize ridge resorption and allow placement of an implant that satisfies aesthetic and functional criteria.[4,5]

Bone grafting is possible because bone tissue has the ability to regenerate completely, with the grafting material ideally enhancing the natural process of osteogenesis. As host bone grows, it will generally replace graft material completely, assisted by new bone growth from vital osteogenic cells resulting in a fully integrated region of new bone.[6]

This natural process of osteogenesis is supported by two distinct processes, namely osteoconduction and osteoinduction. Osteoconduction occurs when bone graft material serves as a scaffold for new bone growth by the host bone. Osteoblasts from the margin of the grafting site utilise the bone graft material as a framework upon which to spread and generate new bone. Osteoinduction, on the other hand, involves the stimulation of osteoprogenitor cells to differentiate into osteoblasts, leading to new bone formation – described by Marshall R Urist in a study done in 1965.[7] This process is facilitated through Bone Morphogenetic Protein (BMP), a growth factor bonded to cell surface receptors that stimulates mesenchymal cells to differentiate into osteoblasts.[8-11] Growth factor enhanced grafts are produced using recombinant DNA technology.[6] They consist of either human growth factors or morphogens (BMPs coupled with a carrier medium, such as collagen).

Different types of grafting material exist namely autograft, allograft, xenograft and alloplastic material. Autograft comprises of autogenous tissue transplanted from one site to another site in the same individual. Autografts possess osteoconductive, osteoinductive and osteogenic properties – as long as it includes bone marrow and sufficient blood supply in the transplant site.[6] Because it fulfils these three basic requirements of bone regeneration, autogenous bone grafts are considered the gold standard in bone regenerative procedures. Limitations involving autogenous bone

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grafting - such as the need for second surgery for harvesting, significant donor site morbidity, limitations in quantity of bone and the potential for complications, have led to the search and study of alternative materials.[10]

Allograft refers to tissue grafts that originate from genetically different donors of the same species, of which Demineralised Freeze-Dried Bone Allograft (DFDBA) is a common example.[6] DFDBA undergoes sterilisation and deactivation of proteins normally found in healthy bone and is commercially available in different formulations such as blocks, matchsticks, conical shapes and particulate form, commonly known as bone sugar.[3] It involves a process of demineralisation with an agent such as hydrochloric acid, whereby calcium and phosphates are removed, but the osteoinductive extracellular matrix is left - which consists mainly of non-structural proteins, including growth factors such as BMPs and type 1 collagen. Apart from being osteoconductive, allografts may therefore also have some osteoinductive properties, although these osteoinductive properties may vary significantly between products from different bone banks due to different manufacturing processes.[12,13]

Xenograft refers to grafts harvested from a donor of a different species, such as bovine, porcine or equine. It is chemically processed in a specific way, resulting in a product with osteoconductive properties, but lacking osteoinductive and osteogenic properties.[3,6,14]

Alloplastic graft material is purely synthetic, for example hydroxyapatite or tricalcium phosphate.[2,15] Similar to xenografts, these grafts are also osteoconductive without osteoinductive or osteogenic properties.

Bone graft material that has both osteoconductive and osteoinductive properties such as DFDBA may therefore serve as both a scaffold for existing osteoblasts and initiate the formation of new osteoblasts, theoretically promoting faster integration of the graft. Histologic studies focusing on the healing patterns of dental extraction sockets after 16 to 20 weeks of healing, comparing commercially available DFDBA to mineralised grafting materials (FDBA), have shown that DFDBA results in greater vital bone gain (28% to 53%) than FDBA (17% to 27%) after three to six months. These properties and the fact that DFDBA is very reasonably priced and easily obtainable, makes it an attractive method of bone grafting during implant placement.[16]

However, because grafting may introduce added risks of post-operative complications and greater cost to the patient, while benefits are not ensured, it is necessary to determine if DFDBA adds value to the bone healing processes related to implant placement.

The objective of this study was therefore to ascertain whether there is any advantage in augmenting dental extraction sockets with DFDBA, by utilising a technique of grafting sockets with DFDBA in combination with a collagen membrane (experimental) and comparing it to sockets that were left to heal naturally (control) in the same jaw of the same patient. The generally accepted parameters indicating new bone formation were used namely a physical count of the number of osteocytes, the quantity of trabecular bone, collagen estimate, inflammatory cell count, blood vessel count and the remaining graft material. Samples of bone from both experimental and control sockets were compared after histological analysis to establish which of the sites displayed a better quality of healed bone, to possibly ensure greater implant stability and better integration.

Method

The study was conducted as a randomised (controlled) clinical trial investigating the histologic difference in bone quality after healing between non-grafted sockets and sockets grafted with DFDBA and a resorbable membrane. The study was conducted by the 1st author as investigator, both in private practice and the School of Dentistry, Faculty of Health Sciences, University of Pretoria (Faculty of Health Sciences Research Ethics Committee approval on 30 June 2016 – Reference nr 231/2016).

Inclusion/exclusion criteria

Basic criteria for selection were patients requiring at least two non-molar extractions, within the same jaw, with planned subsequent dental implant placement. Patients had to be at least 18 years old and given voluntary consent to participate in the study. Single-rooted non-molar teeth due for extraction - with radiological evidence of sufficient bone support and tooth orientation conducive to ideal implant placement, were selected to ensure adequate depth of socket for harvesting of a core biopsy without including surrounding native bone.[4] Multirooted teeth were excluded because of the possibility of interradicular bone being harvested, as well as sockets with a severe dehiscence. Exclusion criteria: impaired immune system, immunosuppressive therapy, uncontrolled systemic disease, anti-inflammatory drug therapy, history of allergy to DFDBA or collagen membranes, teeth with periapical pathology and extensive bone loss during extraction process.

Clinical protocol

Intra-oral examination, peri-apical and panoramic radiological images and Cone Beam Computerised Tomography (CBCT) scans were performed pre-operatively. If deemed necessary, customised acrylic occlusal stents were fabricated on study models to serve as fixed reference guides for both accurate harvesting of core samples and subsequent placement of implants. Intra-operatively the relevant teeth were removed utilising a low-trauma technique to ensure preservation of socket walls. Root remnants and failed fixed prosthesis were removed in advance.

The random allocation of which sockets to graft with DFDBA and which to leave undisturbed was done by the flip of a coin with the patient as witness, purely because it is and has always been regarded as a simple, unbiased method of deciding between two options and is being used regularly in scientific studies.[20]

In the DFDBA graft group a full-thickness gingival flap was raised to expose both labial and facial aspects of the alveolar

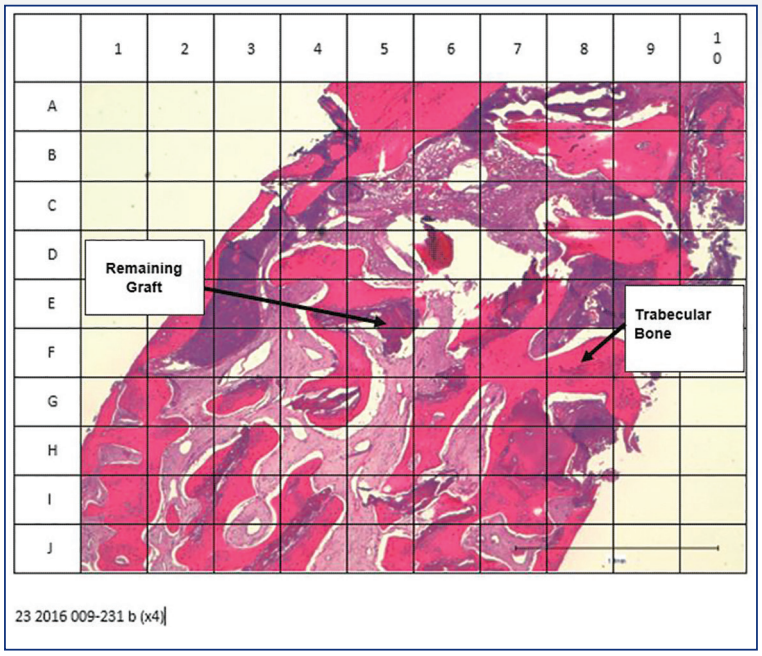

Figure 1: Example of the grid with the imported image (4x magnification).

ridge before commencement of tooth removal. After tooth removal and placement of the DFDBA grafting material, a resorbable collagen membrane was placed to completely cover the socket and extend to a minimum of 3mm beyond the alveolar crest, whereafter the gingival flap was replaced and sutured with monofilament non-resorbable sutures.1 The membrane acts as a barrier against the ingrowth of soft tissue into the healing site while helping to prevent loss of the grafting material at the same time. Current clinical trends tend to favour the use of resorbable membranes, although the study of different types of bone substitution materials combined with different types of membranes is ongoing and their efficacy in obtaining optimal results in immediate extraction socket preservation still needs to be defined.[20] The DFDBA was supplied by the National Tissue Bank of the University of Pretoria (ISO 9001:2000 and ISO 13485:2003) with the collagen membrane being a Jason Membrane (botiss biomaterials GmbH, Germany)

Post-operatively all patients received the same prescription of a 0,2% chlorhexidine rinse twice daily for ten days, the same antibiotic regime of Clindamycin 150mg four times a day for four days and the same analgesics as needed for four days. Clindamycin was chosen due to its effectiveness in both soft tissue and bone infections and also because none of the subjects reported to be allergic to Clindamycin. The analgesic of choice was a standard composition containing 400mg Ibuprofen and 325mg Paracetamol – providing analgesic, anti-inflammatory and antipyretic action.

Sutures were removed after ten days. All cases displayed excellent and uneventful healing at that stage. The quality of bone was assessed 16 to 20 weeks after grafting, as Beck and Mealy, 2010,5 demonstrated that allografted sites did not yield greater bone formation at 24 weeks as opposed to 12 weeks. However, new bone formation is known to be time and subject dependent,[2,4,10] but these variables were eliminated in this study by each patient serving as his own control.

To ensure that only bone from the extraction socket was harvested and also not to compromise primary stability of the implants, at re-entry core samples of at least 8mm (but no longer than 10 mm) in length were harvested by means of a 3,6mm internal diameter trephine - with abundant water supply to prevent overheating of the bone, as the first step in the implant placement drill sequence. The cores were removed from the trephine using a thymosin probe placed into the window of the bur to displace the material. The harvested cores were then stored in a 10% neutral buffered formalin solution in numbered containers. After harvesting of the core biopsies, the final osteotomies were prepared and each of the sites received a dental implant (Neodent, Institut Straumann, Switzerland) with good primary stability established in each case.

Images of each harvested core specimen were digitally captured and examined to differentiate between the parameters indicating new bone formation, as described before.

Data collection

A copy of each slice was printed to check for and eliminate overlaps in order to prevent duplication, resulting in a total of 7200 data containing blocks.

The slices were evaluated for the previously mentioned

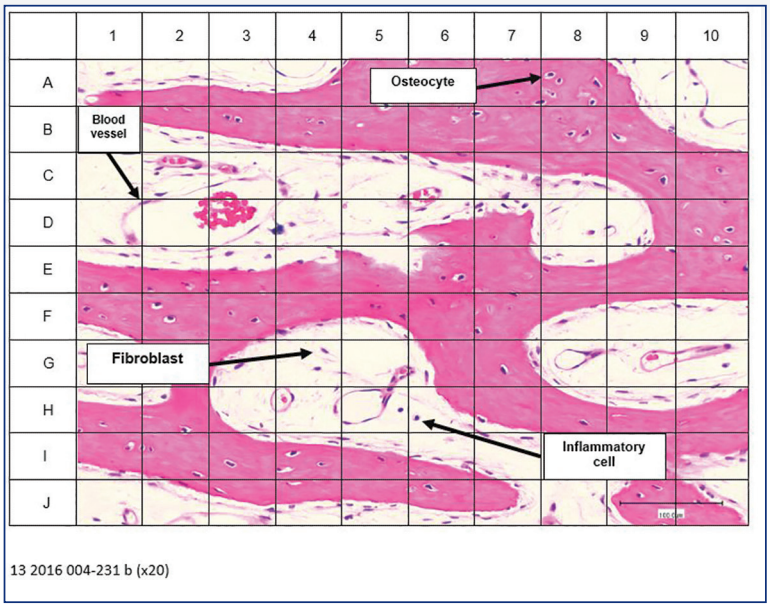

Figure 2: Example of the grid with the imported image (20x magnification).

histological parameters of osteogenesis[15,26,27] by counting the number of osteocytes as well as calculating/estimating percentages of trabecular bone, collagen and RG under 4x magnification and then counting the number of inflammatory cells and blood vessels under 20x magnification.

A case number was assigned, and the location of the implant recorded (tooth number) indicating if it was grafted or not (1 = Yes; 0 = No) . The slide number (typically 4 slides per site, numbered: 1, 2, 3, 4) and grid block number (Figures 1 and 2) were also recorded. Data coverage estimates were done (2 units = 100%; 1 unit = partial coverage (1-99%); 0 = no data) because not all grid blocks were 100% filled with tissue. Inflammatory cells, blood vessels and osteocytes were counted per grid block. The prevalence of collagen, trabecular bone and remaining graft were also subjectively estimated as an absolute percentage and recorded in coverage categories (0 = none; 1 = ≤33.3%; 2 = >33.3%-66.7%; 3 = >66.7%). The co-author controlled the integrity of the datasheet and the primary investigator corrected a minority of initial input errors through recounting. After all the counting was concluded, inflammatory cell, blood vessel and osteocyte counts were summed per site. The categorical estimates (0, 1, 2 and 3) for collagen, trabecular bone and remaining graft were totalled, using the "Countif" function in Excel that enabled the calculation of percentage distributions for each category. The percentage distributions were in turn used to calculate a total estimate for each case, using the numerical midpoint of each category as the utility weight. This method will be referred to as Estimate 1. In addition to this the mean score of the collagen, trabecular bone and remaining graft subjective percentage estimates by the primary investigator were recorded as the second value in this regard. This method will be referred to as Estimate 2. It was decided to use two different methods to estimate the prevalence of tissue types because of the subjectivity of the measurement and the lack of any existing methods that can perform this measurement objectively. It can be argued that if there is strong correlation between the two different ways of measurement then it would indicate that there is some reliability in the methods.

Sample identification was done by the primary investigator and randomly controlled by the supervisor. Inter-examiner reliability testing: The primary supervisor of this project repeated the counts and estimates of 72 randomly selected grid blocks. The Random function in Microsoft Excel was used to isolate the 72 records. The primary investigator of this project repeated the counts and estimates of 72 selected grid blocks that was identified using the same methods as described, above. photo-documented using a Leica DMD108 (DMD= DigitalMicroimagingDevice) Microscope (Leica, Germany) and the best of the two was then utilised to conduct the rest of the study. Some of the slices tore and folded quite considerably during processing and were therefore discarded. Four images (with a scale bar) of each slice were captured: three under 4x magnification - one from each extremity and one from the centre, covering the whole of the sample and one under 20x magnification from the centre of the core, providing a total of 80 digital images.

To count the osteocytes (under 4x magnification) and inflammatory cells (under 20x magnification), the grid method was opted chosen. A 10x10 grid (100 blocks per

grid) was created in Microsoft Word and each digital image was imported into the grid and numbered according to the unique study number allocated to each patient (Figure 1 and Figure 2). Using the scale bar (= 1mm) as reference, the size of one grid block of the 4x magnification slices was calculated to be 0.080mm² (Figure 1). Each block of the 20x magnification slides (scale bar = 100µm) was calculated to be 3 071,75µm² (Figure 2).

Data analysis

The data analysis consisted of descriptive statistics to compare differences in counts between grafted and non-grafted sites and paired t-tests or appropriate non-parametric equivalent analyses (Wilcoxon Sign Rank Test) to establish statistical significance. Significance was set at 0.05. The inter-class correlation coefficient was used to report the intra- and inter-rater agreement.

Results

Eight patients requiring at least two non-molar extractions in the same jaw and one patient requiring three non-molar extractions in both upper and lower jaws, were finally selected to participate. This sample yielded ten sites for natural healing (control) and ten sites grafted with DFDBA and a collagen membrane (experimental). Upon re-entering of the sites, one of the subjects (Subject 7) produced only connective tissue in the coronal 8mm of the non-grafted site. The histological data of subject 7 was eliminated from the study. This resulted in a total of nine grafted and nine non-grafted sites. Six subjects had two sites each and one subject had six sites (four maxillary and two mandibular) totalling 18 sites.

The total sample comprised one male and seven females with ages ranging between 30 and 68, with a mean age of 54,87.

The 18 sites were histologically analysed with nine biopsies in each group. The DFDBA grafted group consisted of two maxillary canines, one maxillary second premolar, one mandibular first premolar and five mandibular canines, whereas the non-grafted group consisted of one maxillary central incisor, one maxillary canine, one maxillary second premolar, one mandibular first premolar and five mandibular canines. The majority of sites (twelve) were from the mandible and the balance (six) from the maxilla.

Clinically, there was no loss of graft material at the four-week follow-up appointments and all the sites healed without complication.

An erratic pattern emerged with no conclusive link between estimated collagen percentages for grafted and non-grafted sites (Table 1, Wilcoxon Sign Rank Test: P=0.0594). Table 1 also illustrates that six of the sites displayed between 5% and 15% less trabecular bone in the grafted sockets, two of the sites displayed 1% and 6% more trabecular bone in the grafted sockets and one site displayed zero difference. These results were however not statistically significant (Wilcoxon Sign Rank Test: P=0.051).

Table 2 showed varying patterns of osteocyte prevalence without any direct gradient leaning towards grafted or non-grafted sites (Wilcoxon Sign Rank Test: P=0.441). Table 2 also showed that in most instances there were more inflammatory cells present when a graft was placed. These differences were however not statistically significant (Wilcoxon Sign Rank Test: P=0.051).

Remaining graft material were less than 5% for all sites (ranging from 0 to 4%)

Discussion

Dental implant treatment aims to restore form and function of the dentally compromised patient when used to support over-structures. Sufficient volume and quality of bone is necessary for anchoring the implant. While the goal of DFDBA placement in extraction sockets is to preserve the volume of bone available for implant placement,[1,2,5,6,12,13,15] it is important to determine the quality of bone achieved through this grafting procedure.[1,13] Based on this premise, this study therefore aimed to histologically compare dental extraction sites grafted with DFDBA with non-grafted sites before implant placement. The comparison was done by assessing the parameters of osteogenesis: number of osteocytes, percentages of trabecular bone, collagen and remaining graft material, the number of inflammatory cells and blood vessels[7,22,23]

It is pertinent to note that this study could not show a meaningful statistical difference for the six histological parameters of osteogenesis between grafted and non-grafted sockets. It stands in contrast to the reportedly osteoinductive properties of DFDBA. A study by Schwarz et al in 1996[13] showed that there could be major differences in DFDBA preparations produced by different commercial bone banks and their ability to induce new bone, due to the use of various bone processing methods. Factors such as particle shape and size, the pH of the solution and varying types and levels of BMPs have been studied and shown to have an influence on the degree of osteoinductive nature of different DFDBA products. [6,18,24,25]

Table 2 showed more inflammatory cells in grafted areas compared to non-grafted areas. Although these differences were not statistically significant (Wilcoxon Sign Rank Test: P=0.051), such gradients are not surprising and can be interpreted as an indicator of the response of the human body to the introduction of foreign material. Higher sample size may have rendered statistically significant results.

Moreover, it was shown that only between 1% and 4% of graft material remained after 16 to 20 weeks, indicating that almost all of the DFDBA had been replaced by trabecular bone, which correlates with time frames suggested by Beck and Mealy, 2010.[25] The outcome of this study showed that at 16 to 20 weeks after extraction, most graft material had been replaced by bone with no additional benefit in terms of bone quality. This finding is consistent with the findings of a randomised control trial reported by Brownfield and Weltman in 2012.[12]

Table 1: Pairwise comparison of collagen and trabecular bone estimates for sites where a graft was placed, or not.

CASE	SITE ID	GRAFT	COLLAGEN % ESTIMATE 1 (DIFFERENCE)	COLLAGEN % ESTIMATE 2 (DIFFERENCE)	TRABECULAR BONE % ESTIMATE 1 (DIFFERENCE)	TRABECULAR BONE % ESTIMATE 2 (DIFFERENCE)
1	11	No	31	29	47	45
1	13	Yes	28 (-3%)	26 (-3%)	36 (-11%)	36 (-9%)
2	33	No	9	8	55	55
2	43	Yes	21 (+12%)	20 (+12%)	40 (-15%)	38 (-17%)
3	33	No	49	48	39	36
3	43	Yes	47 (-2%)	47 (-1%)	27 (-12%)	26 (-10%)
4	33	No	37	34	42	40
4	43	Yes	32 (-5%)	29 (-5%)	37 (-5%)	35 (-5%)
5	43	No	43	29	36	35
5	33	Yes	37 (-6%)	35 (+6%)	36 (0%)	34 (-1%)
6	43	No	29	25	34	32
6	33	Yes	28 (-1%)	25 (0%)	35 (+1%)	33 (+1%)
7	13	No	72	82	1	1
7	21	Yes	36 (-36%)	35 (-47%)	36 (+35%)	34 (+33%)
8	13	No	28	24	38	36
8	23	Yes	22 (-6%)	20 (-4%)	44 (+6%)	43 (+7%)
8	15	No	33	32	41	38
8	25	Yes	36 (+3%)	35 (+3%)	35 (-6%)	33 (-5%)
8	44	No	23	22	48	46
8	34	Yes	27 (+4%)	25 (+3%)	38 (-10%)	35 (-11%)

Note Patient 7 excluded from pairwise comparison
Collagen Estimate 1: Wilcoxon Sign Rank Test: P=0.0594
Collagen Estimate 2: Wilcoxon Sign Rank Test: P=0.594
Pearson Correlation Coefficient for Collagen Estimate 1 and Collagen Estimate 2: (r): 0.967
Median of Collagen Estimate 1 =31%
Median of Collagen Estimate 12 =29%
ICC (Inter-rater agreement): 0.98 (95%CI:0.97-0.99; P=0.000)
ICC (Intra-rater agreement): 0.99 (95%CI:0.99-1.00; P=0.000)
Trabecular Bone Estimate 1: Wilcoxon Sign Rank Test: P=0.051
Trabecular Bone Estimate 2: Wilcoxon Sign Rank Test: P=0.051
Pearson Correlation Coefficient for Trabecular Bone calculated and Trabecular Bone Estimate (r): 0.997
Median of calculated Trabecular Bone Estimate 1=37%
Median of estimated Trabecular Bone Estimate 2=35%
ICC (Inter-rater agreement): 0.94 (95%CI:0.91-0.96; P=0.000)
ICC (Intra-rater agreement): 0.94 (95%CI:0.90-0.96; P=0.000)

The findings of this study should be interpreted with caution. A major limitation of this study is the small sample size. The seven subjects who finished the study were however regarded as a good cross-section of the average patient attending a general dental practice.

It should be noted that the study did not intentionally differentiate between males and females or between upper and lower jaws. The subjects' age was also not taken into account and similar to other studies this study also did not distinguish between smokers and non-smokers. Although these omissions can be considered as limitations it was deemed not necessarily relevant. The idea was to compare grafted to non-grafted sockets within the same individual so that the same patient serves as both experiment and control, thereby negating differences between different people such as smoking, age and gender.

Although it was not intended as part of the study and the study was not designed to evaluate ridge preservation per se, the subjective clinical observation at the time of harvesting and implant placement was however that the grafted sockets were better preserved in terms of the volume and "feel" of the bone – confirmed by the results of various studies.[1,2,5,6,10,1213,15] This phenomenon greatly facilitates the placement of implants without the need for secondary

Table 2: Pairwise comparison of osteocytes and inflammatory cells counted for sites where a graft was placed or not.

				Osteocytes			Inflammatory cells			Blood vessels		
CASE	SITE ID	GRAFT	ADJ No OF DATA UNITS	/0,.8MM²	/MM²	Dif	n	/ 0.154mm²	Dif	n	/ 0.15MM²	Dif
1	11	No	176	13,53	169,11		18	0,10	0,67	2	0,01	0,07
1	13	Yes	103,0	14,83	185,44	16,33	33	0,32	2.09 (+1.42	8	0,08	0.51 (+0.44)
2	33	No	106,5	32,65	408,10		0	0,00	0,00	0	0,00	0,00
2	43	Yes	101,5	18,34	229,31	-178,79	20	0,20	1.28 (+1.28)	5	0,05	0.32 (+0.32)
3	33	No	194,0	21,84	272,94		8	0,04	0,27	12	0,06	0,40
3	43	Yes	101,5	13,71	171,43	-101,51	20	0,20	1.28 (+1.01)	5	0,05	0.32 (-0.08)
4	33	No	116,0	26,38	329,74		26	0,22	1,46	7	0,06	0,39
4	43	Yes	106,0	20,61	257,67	-72,07	3	0,03	0.18 (-1.28)	4	0,04	0.25 (-0.14)
5	43	No	184,5	17,63	220,39		7	0,04	0,25	9	0,05	0,32
5	33	Yes	139,0	18,76	234,44	14,05	9	0,06	0.42 (+0.17)	14	0,10	0.66 (+0.34)
6	43	No	73,0	19,97	249,66		18	0,25	1,61	6	0,08	0,54
6	33	Yes	81,0	14,93	186,57	-63,09	67	0,83	5.39 (+3.78)	5	0,06	0.42 (-0.12)
7	13	No	75,5	0,20	2,48		0	0,00	0,00	0	0,00	0,00
7	21	Yes	164,0	16,80	210,06	207,58	0	0,00	0,00	0	0,00	0,00
8	13	No	109,0	14,44	180,5		2	0,02	0,12	1	0,01	0,06
8	23	Yes	109,0	22,49	271,08	90,58	12	0,11	0.72 (+0.60)	8	0,07	0.48 (+0.42)
8	15	No	98,5	21,26	265,74		6	0,06	0,40	6	0,06	0,40
8	25	Yes	128,0	20,36	254,49	-11,25	29	0,23	1.48 (+1.08)	13	0,10	0.66 (+0.26)
8	44	No	143,5	27,02	337,8		11	0,08	0,50	6	0,04	0,27
8	34	Yes	106,5	30,08	376,06	38,26	23	0,22	1.41 (+0.91)	3	0,03	0.18 (-0.09)

Note Patient 7 excluded from pairwise comparison
Osteocytes: Wilcoxon Sign Rank Test: P=0.441
Median of osteocytes counted/block=19.364
ICC (Inter-rater agreement): 0.97 (95%CI:0.95-0.98; P=0.000)
ICC (Intra-rater agreement): 1.00 (95%CI:0.99-1.00; P=0.000)
Inflammatory cells: Related Samples Wilcoxon Sign Rank Test: P=0.051
ICC (Inter-rater agreement): 0.81 (95%CI:0.50-0.93; P=0.000)

augmentation procedures and is possibly the main reason why so many clinicians routinely perform socket grafting at the time of extraction.

It should be noted that the primary researcher is not a trained histopathologist, but was however advised by a highly skilled specialist oral pathologist and supervised by an experienced specialist periodontist. Reasonable intra and inter-rater agreement was achieved, ranging from "good" agreement for inflammatory cells and blood vessels and "excellent" agreement for the other indicators.[29] It should be noted that there were one or two blinded recounts, under instruction of the co-author as statistician, by both the primary researcher and research supervisor to achieve adequate inter-rater agreement.

Conclusion

This study compared bone quality of naturally healing sockets to sockets grafted with DFDBA. Histologically, mainly by assessing osteocyte counts, percentage of trabecular bone formation and percentage of collagen/connective tissue, no statistical differences could be found between the grafted and non-grafted sites.

Within the limitations of this study, the findings therefore do not support the use of DFDBA in socket grafting to improve the quality of bone.

References

1. Iasella JM, Greenwell H, Miller RL, Hill M, Drisko C, Aziz AB et al. Ridge preservation to extraction alone for implant site development: a clinical and histologic study in humans. J Periodontol. 2003 Jul;74(7):990-99.

2. Kakar A, Rao BH, Hegde S, Deshpande N, Lindner A, Nagursky H et al. Ridge preservation using an in situ hardening biphasic calcium phosphate (ß-TCP/HA) bone graft substitute—a clinical, radiological, and histological study. Int J Implant Dent. 2017 Dec;3:25.

3. Irinakis T. Rationale for socket preservation after extraction of a single-rooted tooth when planning for future implant placement. JCDA. 2006 Dec/2007 Jan; 27(10):917-22.

4. Farina R, Trombelli L. Wound healing of extraction sockets. Endod Topics. 2012(25):16–43.

5. Avila-Ortiz G, Elangovan S, Kramer KWO, Blanchette D, Dawson DV. Effect of alveolar ridge preservation after tooth extraction: a systematic review and meta-analysis. J Dent Res. Published online 25 June 2014, http://jdr.sagepub.com/content/early/2014/06/25/0022034514541127.

6. Prasanna K, Belliappa V, Ghousia F. Bone Grafts in dentistry. J Pharm Bioallied Sci. 2013 Jun; 5 Suppl 1:S125–27.

7. Marshall R Urist. Bone: formation by autoinduction. Science. 12 Nov 1965, 150 (3698),893-99.

8. Borg TD, Mealy BL. Histologic healing following tooth extraction with ridge preservation using mineralized versus combined mineralized-demineralized freeze-dried bone allograft: a randomized controlled clinical trail. J Periodontol. 2015 March; 86(3):348-55.

9. Kini U, Nandeesh BN. Physiology of bone formation. Radionuclide and Bone Imaging. Springer-Verlach (Berlin, Heidelberg): 2012; 29-57.

10. Collins JR, Jiménez E, Martínez C, Polanco RT, Hirata R, Mousa R et al. Clinical and histological evaluation of socket grafting using different types of bone substitute in adult patients. Implant Dentistry. 2014;23(4):489-95.

11. Grover V, Kapoor A, Malhotra R, Sachdeva S. Bone allografts: A review of safety and efficacy. Indian J Dent Res. 2011;22:496.

12. Brownfield LA, Weltman RL. Ridge preservation with or without an osteoinductive allograft: a clinical, radiographic, micro-computed tomography and histological study evaluating dimensional changes and new bone formation of the alveolar ridge. J Periodontol. 2012 May;83(5):581-89.

13. Schwartz Z, Mellonig JT, Carnes Jr DL, De La Fontaine J, Cochran DL, Dean DD et al. Ability of commercial demineralized freeze-dried bone allograft to induce new bone formation. J Periodontol. 1996 Sept;67(9):918-26.

14. Artzi Z, Tal H, Dayan. Porous bovine bone mineral in healing of human extraction sockets. Part 1: Histomorphometric evaluations at 9 months. J Periodontol. 2000, Jun;71(6):1015-23.

15. Giuliani A, Manescu A, Mohammadi S, Mazzoni S, Piattelli A, Mangano F et al. Quantitative kinetics evaluation of blocks versus granules of biphasic Calcium Phosphate scaffolds (HA/ß-TCP 30/70) by Synchrotron Radiation X-ray Microtomography: A Human Study. Implant Dentistry. 2016;25(1):6-15.

16. Wood RA, Mealey BL. Histologic Comparison of Healing After Tooth Extraction With Ridge Preservation Using Mineralized Versus Demineralized Freeze-Dried Bone Allograft. J Periodontol. 2012 March;83(3):329-36.

17. Horowitz R, Holtzclaw D, Rosen PS. A Review on Alveolar Ridge Preservation Following Tooth Extraction. J Evid Base Dent Pract. 2012,Suppl 1:149-60.

18. Evian CI, Rosenberg ES, Coslet JG, Corn H. The Osteogenic Activity of Bone Removed from Healing Extraction Sockets in Humans. J Periodontol. 1982, Feb;81–85.

19. Florencio-Silva R, Rodrigues da Silva Sasso G, Sasso-Cerri E, Simões MJ, Cerri PS. Biology of Bone Tissue: Structure, Function, and Factors That Influence Bone Cells. Biomed Res Int. 2015;421746.

20. Festa VM, Addabbo F, Laino L, Femiano F, Rullo R. Porcine-Derived Xenograft Combined with a Soft Cortical Membrane versus Extraction Alone for Implant Site Development: A Clinical Study in Humans. Clin Implant Dent Relat Res. 2013; 15(5):707-13.

21. Trombelli L, Farina R, Marzola A, Bozzi L, Liljenberg B, Lindhe J. Modelling and Remodelling of Human Extraction Sockets. J Clin Periodontol. 2008;35:630-39.

22. Straumann Extracts. Proceedings of the Fourth ITI Consensus Conference (Group 4: Surgical Techniques in Implant Therapy – Ridge Preservation Techniques for Implant Therapy by Darby I, Chen ST, Buser D. Int J Oral Maxillofac Implants 2009;24 Suppl:260-71).

23. Eskow AJ, Mealey BL. Evaluation of Healing Following Tooth Extraction With Ridge Preservation Using Cortical Versus Cancellous Freeze-Dried Bone Allograft. J Periodontol. 2014 April;85(4):514-24.

24. Zubillaga G, Von Hagen S, Simon BI, Deasy MJ. Changes in Alveolar Bone Height and Width Following Post-Extraction Ridge Augmentation Using a Fixed Bioabsorbable Membrane and Demineralised Freeze-Dried Bone Osteoinductive Graft. J Periodontol. 2003 July;4(7):965-75.

25. Beck TM, Mealey BL. Histologic Analysis of Healing After Tooth Extraction With Ridge Preservation Using Mineralized Human Bone Allograft. J Periodontol. 2010 December;81(12):1765-72.

26. Jurisic M, Manojlovic-Stojanoski M, Andric M, Kokovic V, Danilovic V, Jurisic T, Brkovic B. Histological and Morphometric Aspects of Ridge Preservation With a Moldable, In Situ Hardening Bone Graft Substitute. Archives of Biological Science. 2013;65(2):429-37.

27. Nakahara K, Haga-Tsujimura M, Sawada K, Kobayashi E, Mottini M, Schaller B et al. Single-staged vs. two-staged implant placement using bone ring technique in vertically deficient alveolar ridges – Part 1: histomorphometric and micro-CT analysis. Clin Oral Impl Res. 2016 Nov;27(11):1384-91.

28. Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. Journal of Chiropractic Medicine. 2016 Jun;15(2):155-63.