Comparative Clinical, Radiographical and Biochemical Evaluation of Autogenous Bone Graft in Periodontal Infrabony Defects: A Randomized Clinical Trial

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Abstract

Background: The aim of this clinical study was to evaluate and compare the efficacy of autogenous bone graft procured with the help of bone scraper over open flap debridement in treatment of human periodontal infrabony defects.

Materials and method: 30 infrabony defects were chosen for the study, 15 sites were treated with autogenous bone graft, obtained with the help of scraper and in the rest 15 sites only open flap debridement was performed. The clinical and radiographic parameters were analysed at baseline, 3 months and 6 months using student's t-test. The bone obtained with the help of bone scraper was also analysed biochemically to determine its particle size and Zeta Potential.

Results: At 6 months, there was a significant improvement in clinical attachment level, defect fill and defect resolution in most of the test sites as compared to control. The Zeta potential values showed that autogenous bone thus procured with bone scraper has potential osteogenic activity in the grafted site.

Conclusion: Autogenous bone grafts led to highly significant gain in clinical attachment level, defect fill and defect resolution as compared to open flap debridement.

Key Words: Autogenous bone graft, Defect resolution, Infrabony defect, Open flap debridement, Zeta potential.

Introduction

Periodontal disease is one of the most prevalent dental afflictions worldwide. Periodontitis leads to loss of the tooth-supporting tissues, eventually resulting in loss of teeth. Ideal treatment should involve not only the prevention of further attachment loss, but the regeneration of lost supporting tissues, comprising new alveolar bone and a new periodontal ligament [1]. Various attempts to stimulate periodontal regeneration in the past have focused on filling the defect with some type of material, providing a space or adding growth factors to induce the host cells to form bone [2]. Over the last decades different modalities of regenerative treatment have been developed and applied clinically [3].

Among all the graft materials only autografts and Demineralized Freeze-Dried Bone Allografts (DFDBA) have demonstrated a potential to achieve periodontal regeneration [3]. Historically, autografts were the first bone replacement grafts to be reported for periodontal applications. Autogenous grafts, which are harvested from the patients' own body, are considered the gold standard among graft materials because they are superior at retaining cell viability. These grafts contain live osteoblasts and osteoprogenitor stem cells and heal by osteogenesis. It avoids any risk of rejection or disease transmission. Autogenous bone has osteoconductive, osteoinductive and osteogenic properties [4].

The choice of an autogenous donor site is often based on the amount of bone needed. Extra-oral bone grafts are usually taken from iliac crest, the tibia, or the skull and are used when large amounts of bone are needed. These procedures result in significant morbidity and discomfort to the patient. Conversely, when smaller amount of bone is required, intraoral sites for procuring the graft has several advantages, including reduced operating time & hospitalization as well as lower morbidity and avoidance of cutaneous scarring. But, a second surgical site is still needed. However, the need for second surgical site can be avoided with the use of bone scraper, back action chisel, rongeurs or a bone mill to obtain bone during the surgical procedure [2]. Among all these, bone scraper combines the benefits of both viability and easy procurement over trephines, bone mills or roungers. This hand held instrument (bone scraper) cuts and collects thin shavings of bone from cortical surfaces. The scraper- derived matrix of ribbon like bone shavings and blood has a mortal like consistency and can be easily adapted to the alveolar deficiency to reshape the alveolar crest profile [5].

This controlled clinical study, has focused on the efficacy of autogenous bone graft collected with the help of bone scraper over open flap debridement in treatment of human periodontal infrabony defects. When placed into healthy bleeding bone, the negative- charged surface of autogenous bone graft accelerated the bone-growth cascade. The separation in charge between the solid phase of bone and extracellular matrix creates a potential difference between the solid and the liquid called the zeta potential, which influences the type and nature of proteins and cells harnessed by the surface [6].

Materials and Method

Ethical Clearance and Consent

The protocol was approved by the ethical committee at the Pt. B.D.Sharma, Health University, Rohtak, Haryana, India. The purpose of the study was fully explained to the patients and they were asked to participate by signing written consent.

Study Design

This study utilized a single centered, controlled clinical trial in humans comparing two different therapeutic modalities for the treatment of deep intraosseous periodontal defects. A total of 30 sites, in 15 patients in the age group of 21-64 years (mean 42.5 years), of either sex, suffering from chronic moderate to advanced periodontitis were engaged for the study. One group was treated with a regenerative approach

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i.e. autogenous bone graft (test) and the second with (OFD) without any bone replacement graft. The sites chosen had a radiographic evidence of periodontal interproximal vertical osseous defect ≥ 3 mm, deep of 1-wall, 2-wall, 3-wall or combination type, bone loss from Department of Periodontics and Oral Implantology, D.A.V (C) dental college and hospital yamunanagar, haryana, India. The interproximal vertical defects were selected using clinical and radiographic criteria. The sites were then randomly assigned to test (autogenous bone graft) or control group (open flap debridement). The autogenous bone was harvested with the ebners bone scraper¥ (salvin dental specialists, U.S.A) *(Figures 1-3)*. Patients excluded were those presenting with a history of severe acute or chronic systemic disease, smokers, pregnant or lactating females or having teeth with inadequate endodontic treatments, grade III mobility or a hopeless prognosis. All the clinical measurements were taken with a UNC-15 periodontal probe. A customized acrylic stent was fabricated on the study cast for each patient and the measurements were made from a fixed reference point on the stent.

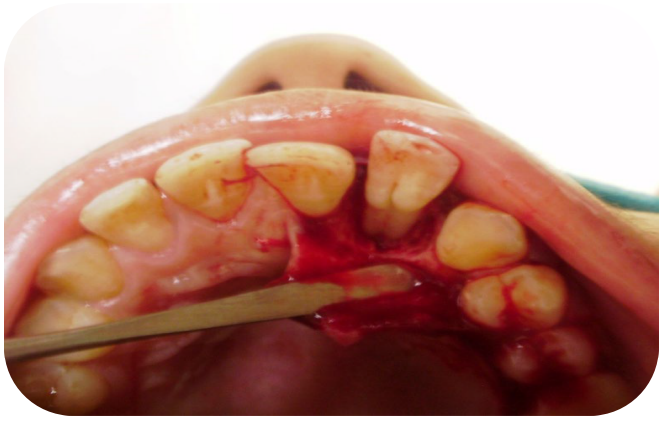

Figure 1: *Photograph showing an osseous defect after debridement w.r.t. maxillary left lateral incisor (mesial and distal).*

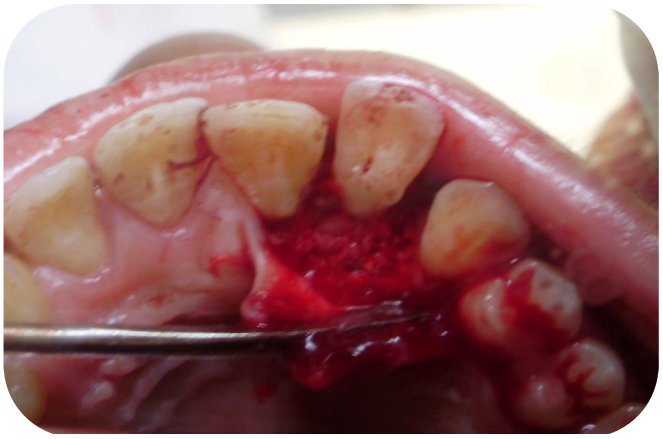

Figure 2*: Photograph showing autogenous bone placed and condensed into the defect.*

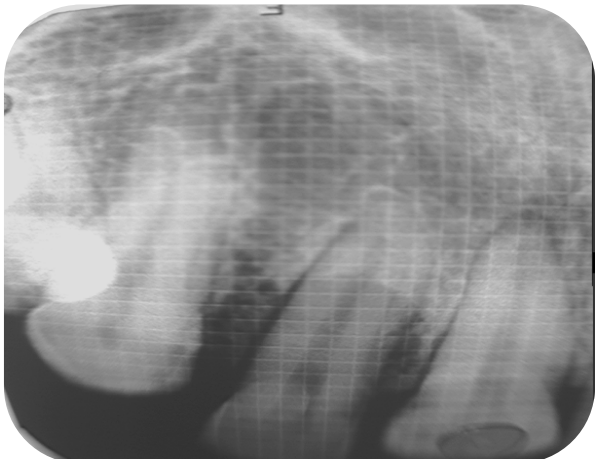

Figure 3: *Photograph showing preoperative radiographic picture of the defect w.r.t. maxillary left lateral incisor.*

Parameters

Pocket depth, clinical attachment level, level of bone defect depth was recorded at baseline. The amount and percentage of defect fill and defect resolution were calculated at 3 months and 6 months post-operatively *(Figure 4)*. Bone particles collected with bone scraper were analysed for size and biological activity determination at Indian Institute of Toxicology Research (IITR), Lucknow.

Surgical Procedure

The surgical procedure was performed utilizing local anesthesia, 2% xylocaine with epinephrine 1:100,000. Intrasulcular incision was given to reflect a full thickness flap well beyond the apices of teeth. Granulation tissues were removed from bony defects, and the exposed root surfaces were scaled and planed with ultrasonic instruments and hand curets. The surgical area was rinsed with sterile saline. Then for test sites, autogenous bone graft, a mixture of bone savings and blood was collected with the help of bone scraper from the area adjacent to the recipient sites. The scraper derived matrix of bone and blood has mortar like consistency and from collecting chamber it was emptied into the dappen dish. It was then adapted to defect site to reshape the alveolar crest profile with the help of plastic filling instrument. Similar procedure was followed at the control sites without the addition of the graft. Flap was then repositioned at the original level and closed with interrupted direct loop sutures using 3-0 Mersilk sutures in the test as well as control sites. Surgical site was protected by applying a periodontal dressing. All the subjects were given both verbal and written instructions as a part of post-operative regimen. After 7 to 10 days dressing, sutures and any plaque present in the area was removed.

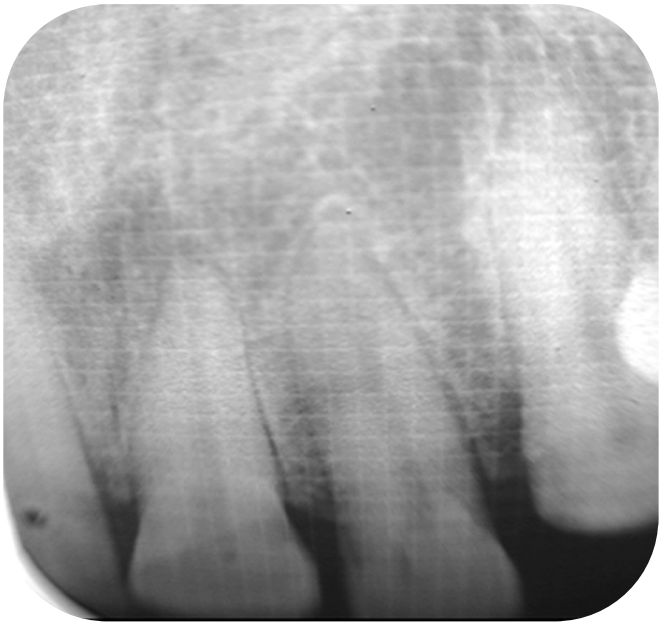

Figure 4: *Photograph showing postoperative radiographic picture of the defect at six months.*

Zeta Potential

The autogenous bone particles were subjected to Dyanamic Light Scattering (DLS) technique to determine size. DLS is also known as Photon Correlation Spectroscopy, which is one of the most popular techniques used to determine the size of particles. The particles subjected to DLS undergo brownian motion and the particle size is determined using the stokes- einstein relationship. The diameter that is measured in Dynamic Light Scattering (DLS) is called the hydrodynamic diameter and refers to how a particle diffuses within a fluid.

Procedure

Samples (0.5mg/ml) were taken in three different solvents i.e., deionized milli quoitent (Mq) water, incomplete culture medium (Minimum Essential Medium; MEM) and complete medium (MEM+10% fetal bovine serum) and coded as P1, P2, P3 for sample. The uniform particle suspension was developed by mixing the samples by pipetting up and down several times. Sample (1 ml) was taken in cuvette and applied inside the instrument for the measurement of Particle size and Zeta potential. Zeta potential was calculated for particles to ascertain their biological activity. It is a function of the surface charge of the particle. It reflects the effective charge on the particles and is therefore related to the electrostatic repulsion between them, zeta potential has proven to be extremely relevant to the practical study and control of colloidal stability and flocculation processes.

Statistical Analyses

For all treatment groups, primary values of continuous ariables were recorded as the mean and standard deviation. Comparison of various parameters among the groups was done using the independent t-test. Data processing and all statistical analyses were performed using a statistical software package.

Results

All the parameters were evaluated at baseline, at 3 and 6 months post-surgically for test sites and for control sites. The statistical analysis was performed using the student t-test. This student t-test was utilized to evaluate and establish differences between two groups (test *vs* control) at baseline, 3 months and 6 months post operatively.

No statistically significant difference was found in mean values of probing depth at baseline (p=0.56), at 3 months (p=0.11) and at 6 months (p=0.49) between test and control group. However, there was a significant gain in mean values of clinical attachment level at 6 months from baseline in test group *(Table 1)*.

The mean amount of defect fill and defect resolution were noted to be statistically significant at 3 months and 6 months (p=0.00) between the test and control sites. Also, the mean percentage of defect resolution at 3 months and 6 months in test sites was 43.22%and 67.35%and 18.8%and 28.8% for control site, which was statistically significant (p=0.00) *(Table 2)*.

The particle size in complete medium was 116.4 nanometer and Zeta Potential of particles ranged from -8.89 to -27.5 mV *(Table 3)*.

Discussion

The present study evaluated the degree of clinical regeneration of deep infrabony defects following the use of autogenous bone graft harvested intraorally with help of bone scraper and compared its efficacy with open flap debridement. Autogenous grafts are considered as gold standard among graft meterials because they are superior at retaining cell viability [7].

Osteoproginitor cells or preosteoblasts proliferate and bridge the gap between graft and the recipient bone. These

Table 1: *Comparison of mean values of clinical changes at baseline, 3 months and 6 months between test and control.*

Pocket depth			
	Test	**Control**	**p-value**
	Mean ± SD	**Mean ± SD**	
Baseline	7.20 ± 0.94	7.00 ± 0.75	0.56
3 months	4.26 ± 1.16	3.60 ± 0.81	0.11
6 months	2.80 ± 0.56	2.66 ± 0.48	0.49
Clinical attachment level			
	Test	**Control**	**p-value**
	Mean ± SD	**Mean ± SD**	
Baseline	6.93 ± 0.96	7.06 ±0.70	0.66
3 months	4.76 ± 1.54	4.33 ± 0.97	0.57
6 months	3.00 ± 1.13	3.13 ± 0.51	0.68

Table 2: *Comparison of mean values of radiographic changes at baseline, 3 months and 6 months between test and control.*

Amount of defect resolution			
	Test	**Control**	**p-value**
	Mean ± SD	**Mean ± SD**	
Baseline	3.76 ± 1.79	3.60 ± 1.41	0.78
3 months	1.66 ± 1.20	0.60 ± 0.20	0
6 months	2.36 ± 1.36	0.96 ± 0.54	0
Percentage of defect resolution			
	Test	**Control**	**p-value**
	Mean ± SD	**Mean ± SD**	
3 months	43.22 ± 24.63	18.8 ± 7.53	0
6 months	67.35 ± 15.38	28.8± 13.15	0

Table 3: *Average size and Zeta potential of the sample.*

Z-Average (Size-d.nm)			Zeta potential (mV)		
Water (P1)	**Incomplete medium**	**Complete medium**	**Water**	**Incomplete medium**	**Complete medium**
	(P2)	(P3)	(P1-Zeta)	(P2-Zeta)	(P3-Zeta)
409.4 nm	605.1 nm	116.4 nm	-27.5 mV	-8.89 mV	-10.8 mV

precursor cells are thought to be hetrogenous, with varying levels of differentiation and corresponding differences in how they respond to biologic growth factors. Transplanted osteocytes usually die in response to anoxia and the surgical injury. Transplanted osteoclasts, however can survive transplantation and these may initiate the resorption of the graft [7]. As the graft material necroses, it releases substances that may stimulate further bone formation. Finally, non viable cellular elements within the graft may act as a scaffold for new bone formation [8].

One limiting factor in the treatment of patients requiring bone grafts has been the inadequacy of amount of bone that can be obtained in a minimally invasive manner. The specially designed bone scraper was used to collect bone particulates in the form of bone shavings. The mean size of these bone shavings was about 1.3mm in length, 200µm in width and about 100µm in thickness [9]. The proposed harvesting technique may have resulted in reduced donor site morbidity and reduced harvesting time compared with the standard block-harvesting procedures. Moreover, due to its mortar like consistency it can be molded with any flat surface instrument, such as a plugger or curette, and it remains where positioned [5,10].

This composite matrix of ribbon-like shavings, with the patient's blood occupying the interconnected porosity, has several potential advantages to promote rapid healing response. Because only a thin layer of bone is planed from the surface, the defect and the subsequent morbidity at the donor site are minimized, and only minimal blood oozing is noted. The potential of cell survival is also enhanced by the minimal cutting temperatures generated with a manual instrument [11].

Controls are needed to eliminate the alternate explanations of the experimental results and eliminate the bias related to researcher bias, environmental changes and biological variations. In experiments involving a surgical procedure, like our study a sham operated group is used to ensure that the data reflects the effect of experiment itself and are not a consequence of the surgery [12]. The subjects were divided into two groups among which 15 sites were taken as test (autogenous bone graft) and 15 were taken as control.

In our study, there was statistically significant gain in mean values of clinical attachment level of 3mm after 6 months for test sites. There was a significant reduction in the pocket depth from baseline to 6 months in the test group. This is in accordance with the results found by Hiatt et al. [11], Froum et al. [13].

The Zeta Potential (ZP) of the bone particles harvested with bone scraper ranged from -8.89 to -27.5 mV which showed that particles were in active state and may respond to growth stimulating factors. Based on this concept, the surface of the material will be charged negatively in an aqueous environment. A number of studies have shown that a material that has an electronegative surface charge (negative zeta potential) is more accessible for the attachment and proliferation of osteoblasts than surfaces with no or even a positive electric charge [15-18]. Cooper and Hunt evaluated the expression of selected osteogenic markers (alkaline phosphatase, osteocalcin, osteopontin, core binding factor alpha-1, and collagen type 1) *in vitro* by reverse transcription-polymerase chain reaction in a culture of osteoblasts in contact with different calcium phosphate materials with positive and negative zeta potential values. They demonstrated a strong correlation of a negative zeta potential with the expression of several osteogenic markers [12]. Other authors reported the significance of relative zeta potentials of bone and different biomaterials and their influence on protein adsorption. Further studies are indicated to substantiate the osteogenic potential of autogenous bone graft.

The results of the present study showed that autogenous bone obtained with a bone scraper improves the healing outcomes regarding probing depth reduction, gain in clinical attachment, osseous defect fill as compared to open flap debridement. The manual collection tool (bone scraper) furnishes autologous bone, avoiding the need for traditional incision-based techniques and cortico-cancellous bone block harvesting with associated post-operative discomfort [9]. Furthermore, the bone material obtained by the means of collector is already in a particulate state, thus reducing operation time and the probability of contamination, since in this case there is no need of use of bone crushers [2,19].

Conclusion

The results of present study seem to support the use of autogenous bone graft in the treatment of infrabony defects. A six months period may be considered too short to evaluate the effect of grafting techniques, biomaterials or other periodontal therapy. However, because of the high resorption rate of the autogenous bone grafts, a longer observation interval may be needed to confirm the stability of the clinical outcome.

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