Charles University in Prague
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Doctoral study programme in Dentistry

Zhodnocení léčby parodontálních kostních defektů kompozitním syntetickým materiálem Fortoss® Vital .

Evaluation of a composite synthetic bone substitute material Fortoss® Vital in the treatment of periodontal intrabony defects

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DECLARATION

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PREFACE

This work has been carried out at the Department of Dentistry, Faculty of Medicine in Hradec Králové, Czech Republic. The purpose of this dissertation is to demonstrate a newer synthetic bone replacement graft material for the treatment of periodontal intrabony defects. This material (Fortoss$^®$ Vital, Biocomposites, Staffordshire, UK) which is a comparatively newer biphasic calcium composite material composed of a porous beta tricalcium phosphate and calcium sulphate is being used in the treatment of periodontal intrabony defects in our dental clinic since the year 2003. This material has the benefit of being both a graft material and an integral membrane produced within one mixture, thereby aiding in guided tissue regeneration.

This dissertation should be of interest to periodontists in Czech Republic and worldwide in general as the work described in this is easier to perform, cost effective and imitates the most commonly used regenerative technique termed as guided tissue regeneration.

I am indebted to many people for the successful completion of this document. Though the following dissertation is an individual work, I could never have reached the heights or explored the depths without the help, support, guidance and efforts of a lot of people.

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SOUHRN

Úvod. Aloplastické kostní štěpy se široce užívají v současnosti v kombinaci s membránami, což zajišťuje realizaci řízené tkáňové regenerace při léčbě nitrokostních parodontálních chobotů. Tato studie byla určena k hodnocení klinických výsledků kompozitního materiálu beta trikalcium fosfátu v kombinaci s kalcium sulfátem při léčení kostních parodontálních chobotů. Kombinace uvedených matriálů umožňuje realizaci řízené tkáňové regenerace.

Metoda. Celkem 47 kostních defektů u 26 pacientů bylo léčeno preparátem Fortoss® Vital (Biocomposites, Staffordshire, UK). Pacienti byli sledováni po 2 roky. Klinické parametry hodnocení zahrnovaly změny hloubky parodontálních chobotů, úroveň gingivodentálního spojení, gingivální recesy,přítomnost či absenci dentálního plaku, BOP na začátku (před operací) a za 2 roky po operaci.

Výsledky. Po chirurgickém ošetření se zmenšila hloubka parodontálních chobotů, zvýšila se úroveň gingivodentálního spojení. Redukce hloubky parodontálních chobotů poklesla po 1 a 2 letech od operace o $1,97 \pm 1.15$ mm ($p < 0,0001$) a $2,07 \pm 1,14$mm ($p < 0,0001$), úroveň gingivodentálního spojení stoupla o $1,68 \pm 1.12$ mm ($p < 0,0001$) a $1,93 \pm 1,36$mm ($p < 0,0001$), gingivální recesy se zvětšily o $0,30 \pm 0.71$ mm ($p = 0,009$) a $0,14 \pm 0,73$mm ($p = 0,571$). Procento plošek s plakem a s pozitivním BOP se redukovalo významně za 2 roky po operaci ve srovnáním s vyšetřením před operací.

Závěr. Léčba parodontálních kostních chobotů kombinací beta-trikalcium fosfátu a kalcium sulfátu vede k signifikantnímu zlepšení kostních parodontálních chobotů po dvou letech od operačním zákroku. Pro přesnější dokumentaci efektu tohoto způsobu léčby je potřeba ještě dlouhodobější sledování a rozšíření počtu sledovaných defektů.

SUMMARY

Background Alloplastic bone graft materials are widely been used these days in combination with barrier membranes to achieve guided tissue regeneration in the treatment of periodontal intrabony defects. This study was designed evaluate the clinical outcome of a composite material, beta tricalcium phosphate in combination with calcium sulphate, in the treatment of periodontal intra-bony defects. The combination of these materials is believed to aid in guided tissue regeneration owing to their properties.

Methods Forty seven intrabony defects in 26 periodontitis patients were treated with Fortoss[®] Vital (Biocomposites, Staffordshire, UK). The patients were followed-up for 2 years. Clinical parameters were evaluated which included changes in probing depth (PD), clinical attachment level/loss (CAL) and gingival recession (GR), presence/absence of plaque and bleeding on probing (BOP) at baseline and at one and two years postoperatively.

Results A decrease in probing depths (PD) was noticed in 24 patients out of the total 26 at one year postoperatively. At two years postoperatively, a decrease in PD was found in all patients but one. The number of BOP positive sites in relation to the involved teeth was reduced from 67 (35.64 %) at baseline to 26 (13.83 %) at 1 year and 28 (14.89 %) at 2 years postoperatively. The number of sites with presence of plaque got decreased from 25 (26.60 %) to 15 (15.96 %) and then increased slightly to 18 (19.15 %) during the same interval. The mean differences in measurements between the baseline and one year postoperatively are a reduction of 1.97 ± 1.15 mm ($P = 0.0001$) in case of PD, a gain of 1.68 ± 1.12 mm ($P = 0.0001$) in CAL and an increase of 0.30 ± 0.71 mm ($P = 0.009$) in GR. The mean differences in measurements between the baseline and two years postoperatively are a reduction of 2.07 ± 1.14 mm ($P = 0.0001$) in case of PD, a

gain of 1.93 ± 1.36 mm ($P = 0.0001$) in CAL and an increase of 0.14 ± 0.73 mm ($P = 0.571$) in GR

Conclusions The treatment with a combination of beta tricalcium phosphate and calcium sulphate led to a significantly favourable clinical improvement in periodontal intrabony defects two years after the surgery. A longer-term evaluation and further studies are necessary to completely ascertain the effectiveness of this material, and a larger sample size is needed.

LIST OF ABBREVIATIONS

BMP	Bone morphogenic protein
BOP	Bleeding on probing
CAL	Clinical attachment level or loss
CPITN	Community periodontal index of treatment needs
DFBDA	Demineralised freeze-dried bone allograft
DNA	Deoxyribonucleic acid
EMD	Enamel matrix derivative
FDBA	Freeze-dried bone allograft
Fig.	Figure
GBR	Guided bone regeneration
GR	Gingival recession
GTR	Guided tissue regeneration
HIV	Human immunodeficiency virus
IGF	Insulin-like growth factor
IL	Interleukin

PBI	Papilla bleeding index
PD	Probing pocket depth
PDGF	Platelet-derived growth factor
PG	Prostaglandin
PMN	Polymorphonuclear leukocytes
PTFE	Polytetrafluoroethylene
SRP	Scaling and root planing
Tab.	Table
TGF	Transforming growth factor
TNF	Tumour necrosis factor
WHO	World health organisation

1 INTRODUCTION

Dental plaque-induced periodontal diseases can be divided broadly into gingivitis and periodontitis based on the presence or absence of attachment loss. Gingivitis is presence of gingival inflammation without loss of connective tissue attachment. Periodontitis is the presence of gingival inflammation along with a loss of connective tissue attachment. In other words, periodontitis is the inflammation of periodontium characterized by apical migration of the junctional epithelium onto the root surface with the concomitant loss of connective tissue and alveolar bone.[6] Periodontitis can be considered to result from an imbalance between destruction and repair of periodontal tissues, triggered by bacteria present in periodontal pockets and possibly aggravated by systemic disorders. In fact, the bulk of tissue destruction is caused by host responses to oral bacteria: host cells (both resident and recruited from blood) release enzymes and cytokines in response to bacterial products. [83, 96]Although periodontitis consists of a family of diseases, these diseases do share a common histopathology, manifest similar signs of disease and usually respond to conventional therapy.

In a clinical and therapeutic point of view, periodontitis can be divided based on the extent or distribution in the mouth to localized and generalized, and based on the severity to slight/initial/mild, moderate and severe/advanced. Although there is no strict cut-off point for the division based on the extent, it has been recommended that the distribution of the disease is designated as: localized if less than 30 % of the sites are involved, and generalized if more. In case of the division based on severity, the amount of clinical attachment loss (CAL) is considered thereby designating the severity of periodontitis as: slight/initial/mild = 1 or 2 mm of CAL, moderate = 3 to 4 mm of CAL and severe/advanced = 5 mm or more of CAL.[4]

Periodontitis is one of the two major dental diseases, the other being dental caries, that affect human populations worldwide at high prevalence rates and that results in loss of teeth. Hence the prevention and treatment of periodontitis is of utmost importance in the field of dentistry. Prevention of periodontitis is achieved through promoting healthy lifestyles including good oral hygiene and reducing/eliminating risk factors.

Contemporary periodontal therapy is directed towards controlling the infection, elimination of inflammation and restoring the lost supporting structures to their original form, function and consistency.[60] Infection control can be achieved by proper initial phase periodontal therapy including through scaling and root planning, maintenance and antimicrobial therapy. Once the infection is controlled and etiologic factors are eliminated, the correction of the consequences caused by the disease is considered in order to achieve a better long-term prognosis of the involved teeth. This phase, called the corrective or surgical phase, includes various surgical procedures aimed at treatment of the unresolved periodontal pockets after the initial therapy, advancing loss of attachment, or need for regenerative procedures thereby trying to re-establish a favourable dental-periodontal relationship to improve the prognosis of the individual teeth and oral health in general. Finally, once the cause is controlled and the consequences have been corrected, recurrence of the disease should be avoided by planned careful follow-ups. This phase of the therapy is mainly supportive in nature and is called the maintenance or recall phase.

Non-surgical therapy performed in the first phase may be sufficient to eliminate the signs and symptoms of mild periodontitis. However, many cases or sites with moderate to severe disease often continue to show signs of inflammation after a non-surgical approach. In such cases, surgical treatment is a necessity. The various surgical approaches implemented in the surgical

phase are open flap debridement (OFD), resective flap surgery, mucogingival surgery and reconstructive/regenerative surgery. The ultimate goal in periodontal therapy is the regeneration of periodontal tissues affected by diseases to their original form, function and consistency. Various techniques are attempted by periodontists in order to achieve periodontal regeneration with varying success. An ideal technique would be the one which is easier to perform and cost effective, which reduce the complexity involved in the treatment and which can predict favourable results.

In the present study, we have evaluated the clinical outcome of a technique which is easier to perform, cost effective and imitates the most commonly used regenerative technique termed as guided tissue regeneration (GTR).

1.1 Epidemiology of periodontitis

Periodontitis being one of the most common infectious diseases worldwide, its incidence and prevalence evoke a major interest among dental health professionals as well as the general public. There have been a lot of studies done on the epidemiology of periodontitis. But the literature reveals a distinct lack of consensus and uniformity in the definition of periodontitis within epidemiological studies. There are also numerous differences in the methods used. The consequence is that data from studies using differing case definitions and differing survey methods are not easily interpretable or comparable. Comparison of effect of risk factors (Odds Ratio, Relative Risk) between studies is hard.[24]

A systematic review of the literature discovered that only 15 studies, out of 3472, gave a definition of periodontitis and indicated how it was measured. The criteria for a diagnosis of periodontitis ranged from 3 mm – 6 mm probing pocket depth and for clinical attachment loss (as an indicator of periodontitis) from 2 mm – 6 mm.[146] The reviewed studies used measurements at different sites using different measurement tools. [146] Researchers have historically used an array of clinical signs and symptoms such as gingivitis, bleeding on probing, pocket depth, clinical attachment loss, radiographically assessed alveolar bone loss and even tooth loss, the ultimate endpoint of periodontal disease.[17,89,93] Further complications are posed by the fact that in some studies multiple disease indicators such as pocket probing depth and clinical attachment level, both representing current pathology and cumulative tissue destruction respectively are used.[27]

1.1.1 The prevalence, extent and severity of periodontal diseases

CPITN (Community Periodontal Index of Treatment Needs) was proposed by WHO in the late 1970's as an index to evaluate the periodontal treatment needs of populations.[14] In the later years, CPITN was used most frequently over the world for epidemiologic studies although there are limitations like non-assessment of tooth mobility, furcation defects, clinical attachment loss etc. Initial field studies using CPITN provided informative results.[2] In a large investigation of 11,305 subjects in Hamburg, only 2.8 % were found to exhibit total periodontal health. 9 % had a CPI score of 1, 28 % had 2, 44 % had 3 and 16 percent had 4. Other studies using CPITN have provided similar results.[3, 15, 43, 53, 75, 80]

The prevalence of periodontitis in adult populations has been measured in several studies by means of clinical assessment of periodontal attachment,[16, 26, 88, 89, 91, 154, 155, 161] assessment of alveolar bone level,[121, 145] or a combination of the two.[122, 123] Some studies were cross-sectional, while others were designed as longitudinal or risk assessment studies.

Table 1.1 summarises some important epidemiological studies of the distribution of periodontal disease in different populations around the world. Only major studies using probing depth and/or probing attachment levels are included. These major studies have presented characteristic patterns of periodontal diseases in various populations of different age groups. It is obvious that the criteria for defining disease cases are far from identical and make direct comparisons between studies difficult. However, it is evident that the prevalence of severe periodontitis is confined within a minority of a population studied. Our current understanding of periodontitis from findings of previous studies has led naturally to identification of factors that may play a role in determining disease initiation and progression on an individual or group level.

Table 1.1: Epidemiological studies of the distribution of periodontal disease in different populations around the world

Author, date, country	Methodology	Results
Baelum et al., 1988, Kenya.[10]	Cross-sectional. 1131 Kenyan adults aged 15–65. LOA and PD at 4 sites per tooth of all teeth. Oral hygiene, tooth mobility. Examinations under natural light.	The oral hygiene was poor with plaque on 75-95 % and calculus on 10-85 % of the surfaces depending on age. PD≥4 mm on <20 % of sites irrespective of age. 10-85 % of the surfaces had loss of attachment ≥1 mm. Skewed distribution of CAL and PD≥4 mm and ≥7 mm. Highest extent of CAL at maxillary molars and mandibular incisors.
Baelum et al., 1997, China.[12]	Longitudinal. 398 Chinese adults remained dentate at follow-up. Limited access to care. CAL and PD at 4 sites per tooth. Oral hygiene, tooth mobility.	Extent of CAL≥3 mm and ≥4 mm in 10 years was positively skewed. 21.8 % of sites lost 3+mm, 9 % 4+ mm. Highest extent of LOA at maxillary molars and mandibular incisors. No significant difference in attachment loss with other populations from developed countries.

Beck et al., 1990, United States.[16]	Cross-sectional. 690 community-dwelling older adults aged 65 or over. Full mouth probing at two sites per tooth.	Blacks- 78 % sites with attachment loss, average loss around 4 mm Whites- 65 % sites with attachment loss, average loss around 3.1 mm
Brown, Oliver & Löe, 1990, United States.[26]	Cross-sectional. 15,132 employed United States adults aged 18–84. Half-mouth assessment of PD and GR at mesial and buccal sites. BOP.	BOP: 44 % of subjects. PD 4–6 mm: 13.4 % of subjects or 0.6 sites per person and at 1.3 % of all sites.
Mumghamba, Markkanen & Honkala, 1995, Tanzania.[107]	Cross-sectional. 1764 subjects aged 3-84 years. GR and PD at buccal surface of ten index teeth. Plaque, calculus, gingival inflammation, oral hygiene behaviour, smoking.	PD$\geq$4 mm in 8 %; PD$\geq$6 mm in 0.5 %. GR$\geq$4 mm in 13 %. Age, male sex, lower educational status, rural residence plaque and calculus, were significantly related to PD and GR in multivariate models.

Slade & Spencer, 1995, Australia. [154]	Cross-sectional. Total 801 subjects 60+ years of age, randomly selected in South Australia. Full mouth. PD and GR measured at three sites per tooth.	CAL 4+mm at one or more sites in 89.1 % of subjects. 78.1 % sites per person had CAL of 2+ mm. Mean CAL: 3.09 mm. Highest mean CAL at maxillary molars. PD is higher than GR in maxilla and equal to GR in mandible. Men had more CAL than women
Söder et al., 1994, Sweden. [155]	Cross-sectional. 1,681 subjects aged 31–40. Full mouth, 6 sites per tooth assessment for PD.	4.9 % had 1 tooth, 6.7 % had 2–5 teeth, 2.4 % had 6–9 teeth and 3.2 % had ≥10 teeth with PD≥5 mm. Calculus, smoking and frequency of dental visits were related to the number of teeth with PD≥5 mm.
Yoneyama et al., 1988, Japan. [172]	Cross-sectional. Random sample of 319 subjects aged 20–79. Mean value, frequency distribution and percentile of PD and CAL at three sites per tooth.	Practically all subjects had one or more sites with periodontal disease. Small subgroup aged 20–59 had advanced disease. Molar teeth expressed more disease. Severity of the disease increased with age.

1.1.2 Tooth and site specificity of periodontal attachment loss

The classic study by Löe et al. (1978) examined different populations and observed different levels of CAL between teeth and sites among individuals irrespective of the populations studied. These differences were clearer with increased age. Mean clinical attachment loss was highest on maxillary molars and mandibular incisors. Buccal and interproximal sites appeared to have different rates of attachment loss as well.[90] Some studies in developing countries have confirmed the unequal or specific distribution between sites on the teeth and between different teeth in a mouth. These studies have also reported highest loss of clinical attachment level on molars in the maxilla and incisors in the mandible.[10, 12]

Several studies using the United States National Institute of Dental Research (NIDR) methodology in populations from developed countries had confirmed the site and tooth specificity of PD, GR and CAL. Slade and Spencer (1995) found among 60 and above years old South Australians the lowest mean CAL in mandibular incisors while confirming that maxillary molars have the highest mean CAL and differences between sites of the extent and severity scores of CAL.[154] A study of the United States employed population (Brown, Oliver & Löe, 1990) also had similar findings.[26] A recent study in a younger population by Thomson, Hashim & Pack, which investigated site and tooth specificity of CAL and its components showed differences between site and teeth. They did not find higher extent and severity scores in mandibular incisors as compared to lower molars, in contrast to findings from developing populations.[161] An important issue to consider when comparing between sites is the distribution of tooth loss by tooth type. Different tooth groups tend to be lost at different frequencies, thus making the comparison of periodontal destruction components sometimes difficult. Molar teeth,

which may accumulate more caries and/or periodontal disease, are more likely to be lost than other teeth. Therefore, a significant proportion of heavily diseased sites of those teeth may be already lost by extraction owing to deep destruction by caries. In this case, remaining teeth may be recorded as having more severe disease compared to missing teeth when it may not be true. A further question which arises is that some proportion of destruction recorded may not be true disease; it may be owing to other non-disease factors such as dehiscence of bone or habits causing gingival recession. The proportion of this destruction may not be equally distributed across the mouth. This issue may contribute to the unequal distribution of disease between teeth and sites. Previous findings suggested the site- and tooth-specificity of patterns of periodontal loss of attachment. However, no inferential testing of statistical significance between these differences had been done. Furthermore, some discrepancies in comparison between sites have been reported in findings of several studies referred to above. It is not clear yet whether these inconsistencies were owing to chance alone or to differences in methodologies, the discrepancies in distribution of tooth loss or to real differences between populations studied. This question needs to be further investigated.

1.1.3 Distribution of periodontal diseases in developed and developing populations

The previously held belief that higher prevalence and severity of periodontitis exist among populations of developing nations where living standards are lower and less access to health care services compared to that of developed nations has not been confirmed by most studies. Studies in the 1960s using composite indices had come to the conclusion that developing nations had poorer oral hygiene status and, consequently, more periodontitis.[141, 142] That conclusion was a

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result of the previously dominant concept of a necessary and sufficient role of oral hygiene in the disease initiation and progression and the scarcity of studies conducted among developing populations. However, Anerud et al. (1983), comparing groups of United States, Norwegian and Sri Lankan young adults found strikingly similar rates of periodontal breakdown, despite the last group having much poorer oral hygiene conditions.[4] Furthermore, Baelum et al. (1996) raised very interesting issues by recalculating and comparing findings from several studies in various countries. Their meta-analysis had shown similarities in the disease patterns in six out of the eight samples, irrespective of oral hygiene conditions and levels of access to dental care.[11] Loss of periodontal attachment data (mostly from developed countries) and the more superficial CPITN data from many developed and developing countries have presented similarities in the prevalence and severity of periodontitis.[11, 13] There are few exceptions from some studies of Sri Lankan tea workers (Löe et al., 1978) and South Pacific Islands (Cutress, Powell & Ball, 1982).[44, 91] However, it is obvious that there are no clear differences in the prevalence of severe stages of periodontitis between developed and developing populations irrespective of methodologies and indices used. Clear differences are only apparent in poorer oral hygiene and greater calculus accumulation in even a young age group in populations of developing countries. Thus, the prevalence and severity of the disease can be considered far more similar between populations and are confined to small groups at high risk in each population. Different populations, however, may differ in the number of risk factors or in level of exposure to a particular risk factor or may have different resistance to risk factors. This area in periodontology requires further research.

1.2 Tissue destruction in periodontitis

The periodontium consists of two hard tissues and two soft tissues. The hard tissues are alveolar bone and the cementum and the soft tissues are gingiva and periodontal ligament. The structure and composition of periodontium are affected in many acquired and heritable diseases, most significantly periodontal diseases. The hallmarks of periodontal disease are bone loss, loss of connective tissue attachment to cementum and destruction of soft tissues. Periodontitis is a major cause of tooth loss in human beings.

The pathogenesis of human periodontitis was first documented in detail by Page and Schroeder in 1976.[118] The general principles and overall conclusions of their research are still valid. As time goes by, more and more researches were done trying to find out the exact mechanism of pathogenesis of periodontitis. In recent years, much has been added to the knowledge of the pathogenesis of the periodontal diseases, not only at the cellular, but also at the molecular and genetic levels. What this has done is that it offered new potential for prediction of risk and for treatment and control of the periodontal diseases. However, much of this field remains to be explored.

The pathogenesis of periodontal disease involves the sequential activation of a great variety of components of the host immune response, primarily acting to defend periodontal tissues against bacterial aggression, but also functioning as mediators of tissue destruction. Pathogenic microorganisms can produce tissue destruction in mainly two ways: (i) by the direct pathological effects of bacteria and their products on the periodontium which induce cell death and tissue necrosis; and (ii) indirectly, through activation of inflammatory cells that can produce and release mediators that act on effectors, with potent pro-inflammatory and catabolic activity.

Some bacteria also interfere with the normal host defence mechanism by deactivating specific antibodies or inhibiting the action of phagocyte cells. The expression of the disease results from the interaction of host, microbiological agents, and environmental factors. Leukocytes play a critical role in the pathogenesis of the disease, producing different cytokines, chemokines, and other mediators, thus generating a host defence response, as well as inducing tissue inflammation and bone destruction. Polymorphonuclear leukocytes (PMNs), which normally provide protection, can themselves contribute to tissue pathology. During the process of phagocytosis, these cells typically "spill" some of their enzyme content extracellularly during a process known as degranulation; some of these enzymes are capable of degrading the surrounding host tissues, namely collagen and basement membrane constituents, contributing to tissue damage. There is increasing evidence that the bulk of tissue destruction in established periodontitis lesions is a result of the mobilization of the host tissues via activation of monocytes, lymphocytes, fibroblasts, and other host cells. Engagement of these cellular elements by bacterial factors, in particular bacterial lipopolysaccharide (LPS), is thought to stimulate production of both catabolic cytokines like Interleukin 1 (IL-1), Interleukin 6 (IL-6), Interleukin 8 (IL-8), and Tumor necrosis factor alpha (TNF-α) and also of inflammatory mediators including arachidonic acid metabolites such as prostaglandin E 2 (PGE 2). Such cytokines and inflammatory mediators in turn promote the release of tissue-derived enzymes, the matrix metalloproteinases, which are destructive to the extracellular matrix and bone.[22, 116] The proportion of damage caused by direct effects of the bacteria and that caused by indirect host response mediated action has yet to be established. Although numerous bacteria can degrade tissue directly, like enzymatic breaking down of extracellular substances like collagen and even host cell membranes, Birkedal- Hansen et al suggested that host connective tissue is mainly degraded by the host.[22] Thus, the loss of

connective tissue is a defence mechanism; the host attempts self-protection by the apical proliferation of junction epithelium, escaping from the toxic root surface to avoid lesion progression.

In the article by Page and Schroeder, four phases of periodontal lesion progression in the cellular level were described: initial, early, established and advanced. The initial lesion was the response of gingival tissues within 2 to 4 days to a beginning accumulation of microbial plaque biofilm with a classic acute exudative vasculitis. This response, which includes loss of perivascular collagen, is comparable to that elicited in most other tissues subjected to acute injury and may be a consequence of the elaboration and release of chemotactic and antigenic substances by microbial plaque. Within 4 to 10 days, the early lesion develops. It is characterized by a dense infiltrate of lymphocytes and other mononuclear cells, pathologic alteration of fibroblasts, and continuing loss of the connective tissue substance. The structural features of the early lesion are consistent with those expected in some form of cellular hypersensitivity, and a mechanism of this kind may be important in the pathogenesis. The early lesion is followed by the established lesion which develops within 2 to 3 weeks and is distinguished by a predominance of plasma cells in the absence of significant bone loss. The established lesion, which is extremely widespread in humans and in animals, may remain stable for years or decades, or it may become converted into a progressive destructive lesion. In the advanced lesion, plasma cells continue to predominate although loss of the alveolar bone and periodontal ligament, and disruption of the tissue architecture with fibrosis are also important characteristics. The initial, early, and established lesions are sequential stages in gingivitis and they, rather than the advanced lesion which is manifest clinically as periodontitis, make up the major portion of inflammatory gingival and periodontal disease in humans.

1.3 Treatment of periodontitis

According to the principles of *lege artis*, all practitioners of the dental profession are obliged to offer treatment based on the most current scientific and clinical knowledge available. The etiology of periodontitis is now well understood, and efficient methods for prevention, treatment, arrest, and control of periodontitis is developed based on that. Periodontal treatment requires long-range planning. Its value to the patient is measured in years of healthful functioning of the entire dentition.

The aim of the periodontal therapy is to eliminate inflammation and the etiologic factors and to regenerate and restore the periodontal tissues affected by diseases to their original form, function and consistency.[60] In order to achieve this, a periodontal therapeutic strategy is needed, planned in various phases. The first phase of treatment consists of controlling the etiological factors, thereby halting the further progression of the disease. This phase can be called as etiologic or hygienic phase and it includes patient motivation and education in matters of oral hygiene, elimination of supragingival and subgingival dental calculus and contaminated radicular cementum and modification/elimination of other plaque retentive features. The standard procedure employed for elimination of subgingival calculus and other unwanted contents of the periodontal pocket is commonly termed as scaling and root planing (SRP). Adjunct local or systemic antibiotics or other chemotherapeutic agents are also used widely. After the first phase of treatment, once the cause of the disease is controlled, the correction of the consequences provoked by the disease is considered. This phase, called the corrective or surgical phase, includes various surgical procedures aimed at treatment of the unresolved periodontal pockets, advancing loss of attachment, or need for regenerative procedures thereby trying to re-establish a

favourable dental-periodontal relationship to improve the prognosis of the individual teeth and oral health in general. Finally, once the cause is controlled and the consequences have been corrected, recurrence of the disease should be avoided. This implies the third phase of the periodontal treatment, also called the maintenance or recall or supportive phase.

1.3.1 Non-surgical therapy

Non-surgical therapy includes both mechanical and chemotherapeutic approaches to minimise or eliminate the primary etiology of periodontitis, the microbial biofilm. Mechanical therapy consists of debridement of the radicular surfaces by the meticulous use of hand or power-driven scalers to remove dental plaque, endotoxins, calculus and other retentive features. The term mechanical therapy refers to supragingival and subgingival scaling as well as root planing. Chemotherapeutic approaches include topical application of antiseptics or sustained-release local drug delivery systems and the use of systemic antibiotics.

Scaling and root planing (SRP) is one of the most commonly utilized procedures for the treatment of periodontal diseases. Scaling and root planning allow reduction in pocket depth mainly by new connective tissue or epithelial attachment; with a probable gain in clinical attachment level. Periodontal literature is sated with studies showing the treatment of periodontitis by scaling and root planing results in reductions of probing depths.[9, 35, 67, 68] The decrease in probing depth is caused partly by the shrinkage of the pocket soft tissue wall manifested as recession of the gingival margin which results from a decrease in soft tissue inflammation; and partly from the gain in clinical attachment.[9, 35, 67, 68] In a thorough evidence-based review published in 1996, Cobb calculated the mean probing depth reduction and gain of clinical attachment that can be achieved with root planing at sites that initially were 4 to 6 mm in

depth and 7 mm or greater in depth. He reported mean pocket depth reductions of 1.29 mm and 2.16 mm, respectively, and mean gains of clinical attachment of 0.55 mm and 1.29 mm, respectively.

Most of the beneficial effects of SRP appeared to occur within the first 3 months with mean attachment levels and pocket depths remaining relatively unchanged at later time points.[120] An increase of clinical attachment refers to new connective-tissue attachment (that is, new periodontal fibres inserting into the cementum) or formation of a so-called long junctional epithelium (repair). Usually, the latter occurs.

1.3.2 Surgical therapy

Non-surgical therapy performed in the first phase may be sufficient to eliminate the signs and symptoms of mild periodontitis. However, cases or sites with moderate to severe disease often continue to show signs of inflammation after a non-surgical approach. In such cases, surgical treatment is a necessity. Many different surgical techniques and materials have been reported in the literature to successfully treat periodontal intrabony defects. The various surgical approaches implemented in the surgical phase are open flap debridement (OFD), resective flap surgery, mucogingival surgery and reconstructive/regenerative surgery. An ideal technique would be the one which could achieve periodontal regeneration and which is easier to perform and cost effective.

 As mentioned earlier, the ultimate goal in periodontal therapy is the regeneration of periodontal tissues affected by diseases to their original form, function and consistency. In teeth in which

continued function requires additional periodontal support, optimal treatment involves not only controlling periodontal infection, but also regeneration of the lost periodontium.

The current techniques in the treatment of periodontitis aimed at periodontal regeneration include open flap debridement- OFD,[47, 55 74, 134] the use of bone grafting materials,[25, 129, 133, 135, 140, 174, 177] Guided tissue regeneration – GTR,[37, 40, 41, 46, 51, 85, 97, 108, 112] and also the use of certain biologic modifiers like Enamel matrix derivatives – EMD[49, 149] or various other growth factors (i.e. Platelet Derived Growth Factor - PDGF, Insulin like Growth Factor – IGF, Transforming Growth Factor-β (TGF-β) including Bone Morphogenetic Proteins – BMPs).[77, 92, 117, 130, 143]

1.4 Periodontal wound healing

The basic events of wound healing are the same regardless of the location of the body. Thus periodontal wound healing after an injury or a surgery also involves three overlapping phases that are independent.[34] The phases can be called as inflammatory phase, proliferative/granulation phase and remodelling/maturation phase. Traumatic or surgical injury causes haemorrhage and extravasation of blood, and a blood clot is formed. The blood coagulation process and activated complement pathway generate many polypeptide mediators, and the blood clot serves as a provisional matrix for the migration of inflammatory cells. In the proliferative phase, re-epithelialisation occurs, along with angiogenesis and activation of various components of extracellular matrix and the clot is replaced by a granulation tissue. During the remodelling phase, the granulation tissue matrix is replaced with fresh connective tissue. A fibrous scar replaces the wound when regeneration is not possible.[34, 153]

Periodontal wound healing is regarded as the most complex healing process in the human body.[98] It is mainly because of the different types of tissues involved and that the healing should take place in an open system which is continuously contaminated with bacteria and their products. Therefore, the healing results following periodontal therapy can be quite variable. In the site of periodontal healing, we have four situations which might occur. First, the epithelium will try to migrate from the wound margin down to the base of the sulcus. If this occurs, the reestablishment of the pocket or in the best scenario, long junctional epithelium will be established. Secondly, the connective tissue will try to grow into the area of the defect. If this occurs, the end result will be external resorption at the connective tissue-root interface. Thirdly, if the bone cells are allowed to repopulate the area of defect, ankylosis or resorption will occur at

the junction of bone to tooth interface. Karring et al in 1980 demonstrated this in beagle dogs, of which when the roots were extracted and transplanted into a surgically created alveolar bone in the edentulous part of a jaw, ankylosis and root resorption occur.[78] Finally, the cells of periodontal ligament, if allowed to repopulate the root surface, the regeneration can be established. Nyman et al in 1982 showed in a study in monkey with the use of millipore filter to exclude the epithelium and the gingival connective tissue. After three months, the histological specimen demonstrated new attachment, new cementum, and new bone.[113] He further confirmed this result with the follow up study on a root surface in human using the principle of GTR. A block biopsy of a lower central incisor at three months after surgery showed new cementum and with inserting collagen fibres extending five millimetres coronally from the apical level of root planning.[115] Melcher in 1976 reported these four tissue compartments in the periodontium and that each of these tissues was capable of producing a unique cell phenotype, and that the type of healing following periodontal therapy depended on the phenotype of the cells which first repopulated the root surface.[100]

In short, healing of periodontal wounds after periodontal therapy can be achieved either by repair or by regeneration. Repair involves only the restoration of continuity in the wound or defect area without regeneration of the originally intact tissues' form and function: e.g. long junctional epithelial attachment. Regeneration of supporting tooth structures is a huge step up in managing advanced periodontal disease and preventing tooth loss. Like other treatment options, it is not a panacea for all patients affected by periodontitis, but research gives us enough evidence to support the use of regenerative therapies in periodontics.

1.5 Guided Tissue Regeneration: Principles and evolution

The ultimate goal of periodontal therapy is predictably regeneration of the periodontal tissues destroyed by peridontitis. Regeneration should be distinguished from repair. Regeneration is defined as the type of healing which completely replicates the original architecture and function of a part. It involves the formation of a new cementum, periodontal ligament, and alveolar bone. Repair, on the other hand, is merely a replacement of loss apparatus with scar tissue which does not completely restore the architecture or the function of the part replaced. The end product of repair is the establishment of long junctional epithelium attachment at the tooth-tissue interface. Traditional therapeutic modalities usually failed to predictably regenerate the periodontal tissue lost due to disease process. The principle of guided tissue regeneration (GTR) can be applied and may result in restitution of the functional periodontal apparatus (new cementum, periodontal ligament, and alveolar bone). Procedures which relies heavily in the principle of GTR involves those whose end result is the complete regeneration of periodontal structures which were lost due to periodontal disease, those whose objective is the ridge augmentation to allow proper placement of osseointegrated implant, and also the procedures which are utilized in treatment of furcation and recession defects.

GTR procedures attempt to achieve periodontal regeneration through biologic principles of differential tissue response. The cells of periodontal ligament if allowed to repopulate the root surface by preventing the faster proliferating epithelial cells and other unwanted cells in to the periodontal bony defect, regeneration can be established. The principle of GTR thus involves the use of a physiological barrier which is placed over the denuded lesions in such a way that all periodontal tissue except the periodontal ligament cells and the alveolar bone are prevented from

reaching contact with the root. The cells of periodontal ligaments are the only ones which seem to have the capacity to form new attachment. Cells of periodontal ligament migrates and differentiate faster than those of bone, thus even though bone cells were allowed to migrate to the area along with the cells of PDL, we would expect the cells of PDL will repopulate along the root surface.

The use of a barrier has first been reported by Younger in the Dental Cosmos of 1904, in which a Japanese paper saturated with liquid celluloid was used to form a protecting wall over the roots and the edge of the gingiva.[173] Prichard in 1957 further stated that cells that are necessary for the genesis of periodontal ligament, cementum, and alveolar bone are available in the area that borders the bony deformity.[128] Melcher in 1976 classified the four tissue types which will repopulate the root surface as described previously.[100] Further investigations in the 1970's and 80's supported Melcher's concept. Caton et al examined healing following four different modalities of periodontal treatment (scaling and root planing, modified Widman flap with debridement alone or in combination of autogenous or synthetic bone graft). The end results demonstrated the establishment of long junctional epithelium between the gingival connective tissue and the root surface upon healing.[31, 32, 33] This finding supported other similar studies that conventional nonsurgical and surgical periodontal therapies usually resulted in repair rather than regeneration.

The effects of epithelial exclusion are further investigated by Nyman in 1980. When root was allowed to contact alveolar bone, ankylosis and root resorption occurred. When root was allowed to contact the gingival connective tissue and the root surface had been denuded of periodontal fibre, the root resorption occurred.[114] These observations suggested that exclusion of gingival

epithelium alone does not promote periodontal regeneration. His further study with the Millipore filter in 1982 reported that the periodontal ligament cells has a considerable potential for periodontal regeneration, and that this potential is manifested only when the gingival epithelium and connective tissues are excluded from the periodontal wound.[113] He further followed up on the human study on the selected mandibular incisor. Again, histological evaluation revealed new cementum with inserting collagen fibres extending 7 mm from the apical level of root planing in a coronal direction.[115]

In 1986, Gottlow et al presented a case report of 12 periodontally involved teeth from 10 patients treated using this biologic principle. Eleven of these teeth formed the experimental group and were treated by flap elevation, granulation tissue debridement, scaling and root planing followed by placement of ePTFE barrier (Goretex membrane). The remaining tooth was also surgically treated but without the placement of barrier as the controls. Clinical results from re-entry indicated significant gain in clinical attachment and probing depth reduction, as well as an apparent bone fill in some of the previously presented osseous defects. Histological observations disclosed a substantial amount of periodontal regeneration in all the teeth treated with the barrier.[66] These findings demonstrated that periodontal regeneration could be predictably obtained in humans by placing the physical barrier, which selectively excludes gingival epithelium and connective tissue and favours periodontal ligament repopulation of the root surface.

The cellular process involved in the development of the periodontium and in wound healing must be understood in order to comprehend the regeneration concept in periodontal defects. The major type of cell in the periodontal ligament is the fibroblast. Fibroblasts are located throughout the

connective tissues of the body, where their role is to maintain the extracellular matrix substance. The periodontal fibroblast is capable of extensive protein and collagen synthesis and that it responds well to the molecular mediators during the process of wound healing. Fibroblast apparently has the potential to develop into different types of cells during wound healing, depending on the molecular mediator that stimulates it. The precursor cells of the periodontal ligament, in this case the fibroblast can differentiate into osteoblasts, or cementoblasts, depending on their position. Cell migration in the periodontal ligament seems to occur starting at the bone interface and continuing along the collagen fibres. There must be a mechanism which selectively activates bone precursor cells to repopulate the area and establish a new tissue exactly like the originating tissue, with each type of cell in its proper position. Specific cellular types that repopulate the wound defect will determine the form and type of tissue that will be created. The proliferation of the proper type of cells in their proper position may be regulated via molecular growth factors which are thought to be responsible for specifically stimulating the proliferation of cementum, periodontal ligament, and bone cells. The ultimate goal of GTR is to use a mechanical barrier to provide the environment necessary for the body to utilize its natural healing potential and to regenerate lost and absent tissue. Ultimately, the efficacy of periodontal membranes in conjunction with wound healing is the result of a combination of different mechanisms- mechanical, cellular, and molecular.

The chief clinical indications for the use of GTR are the class II furcation defects, two or three walled vertical, interproximal, and circumferential intrabony periodontal defects. Class III furcation may be treated with GTR but with less predictability of success. Other clinical indication of GTR are the ridge augmentation (can also be referred to as guided bone

regeneration), and the treatment of gingival recession. Sites which may be at risk for post surgical recession are best treated with nonresorbable barriers, since barrier exposure may accelerate resorbable barrier degradation. Bone graft may be used in combination with GTR for the supporting purpose to prevent the collapse of the membrane. Success of GTR treatment relies heavily on the ability to stabilize the blood clot. Blood clot stabilization is the major prerequisite for the regeneration to evolve. Wikesjö has shown without the blood clot (with the use of heparin to dissolve the clot on root surface), the regeneration failed to occur.[170] Other factors which aided in successful GTR technique are oral hygiene, adequate initial hygienic therapy, proper flap selection and management, adequate debridement to completely remove all granulation and soft tissue at the treated site, the decortication of the bony defect underneath the membrane to stimulate the formation of a blood clot, adequate adaptation of membrane to prevent epithelium to migrate underneath the membrane, adequate debridement to denude the bone of the defect site, adequate size and shape barrier chosen (extending 2-3 mm pass the border of the defect), and finally, complete coverage the membrane underneath the flap upon suturing. Sutures may be removed after 7-10 days. If PTFE sutures are used, they may be allowed to remain for a longer period of time in order to aid in flap adaptation since this type of suture does not cause wicking and trapping of bacteria. If the barrier is nonresorbable, it is usually removed approximately 4-8 weeks. The most important period of cell migration and proliferation are the first 30 days. If the membrane can be maintained underneath the flap for this initial period, we can achieve closely or maximal amount of regeneration. In short, primary wound closure to ensure undisturbed and uninterrupted wound healing, angiogenesis to provide necessary blood supply and undifferentiated mesenchymal cells, space maintenance/creation to facilitate adequate space for

bone ingrowth, and stability of wound to induce blood clot formation and uneventful healing events are desirable characteristics to achieve in any GTR procedure.[162, 168]

1.5.1 Nonresorbable barriers

Nonresorbable membranes retain their build and form in the tissues, requiring a second surgical procedure for removal, thus adding to the trauma of the periodontal tissues and to patient discomfort, as well as raising the costs and duration of therapy. The first non-resorbable membranes approved for clinical use were made of expanded polytetrafluorethylene (ePTFE, Gore-Tex®). PTFE is a fluorocarbon polymer with exceptional inertness and biocompatibility, prevents tissue ingrowth and does not elicit foreign-body response after implantation, but is nonporous.[165] Expanded PTFE is chemically identical, causes minimal inflammatory reaction in different tissues, allows tissue ingrowth and has been used in vascular surgery for several decades.[29, 48, 52] It is manufactured when PTFE is subjected to high tensile stress, forming porous microstructure of solid nodes and fibrils. Gore-Tex® ePTFE membrane consists of two parts. First, an open microstructure collar which promotes connective tissue ingrowth, positioned coronally, and prevents apical epithelial migration and ensures wound stability. This membrane part is 1 mm thick and 90 % porous.[147] The other part is occlusive membrane 0.15 mm thick and 30 % porous, serving as a space provider for regeneration, which possesses structural stability and serves as a barrier towards the gingival flap.[76, 152] Human histological samples have indicated that ePTFE membranes can lead to significant periodontal regeneration after a 3 months healing period.[66] Six months after insertion of ePTFE membrane new cementum with inserting fibres was demonstrated.[38] Effectiveness of ePTFE membranes was investigated in numerous clinical studies. Membrane insertion can cause minor complications such as pain, purulence and

swelling, with an incidence somewhat higher than that reported for conventional periodontal surgery.[109]

Giampaolo Pini Prato and co-workers in 1992 reported the four year follow up results of a clinical trial of which guided tissue regeneration versus mucogingival root coverage surgery were used in the treatment of human buccal recession. The result showed that average reduction in the recession was similar in the two groups while probing depth reduction and clinical attachment level were greater in the GTR group.[125] A study by Roccuzzo and Buser demonstrated a mean root coverage of 84 % when buccal gingival recessions were treated with e-PTFE membranes and miniscrews.[138]

The Gore-Tex® ePTFE membrane has been modified by incorporation of titanium reinforcements, set between two ePTFE layers, resulting in heightened mechanical strength and better space maintenance.[38, 71, 152] Animal studies revealed clinically relevant cementum and bone regeneration 2 months after insertion,[152, 171] and clinical studies found no difference compared to non-modified membranes.[18] Titanium reinforcement membranes also have their application in guided bone regeneration procedures (GBR) aimed at augmentation of toothless alveolar bone, in cases where implants are planned and insufficient alveolar bone mass is present. Membrane made from dense non-porous PTFE-a (TefGen-FD®) was tested on rat calvarial defects showing results similar to ePTFE membrane application, but with limited tissue integration.[42]

In the literature use of other nonresorbable materials for GTR membranes is described, like several case-reports of rubber-dam[39, 144] and glass ionomer.[1] Although the number of

investigations is limited, it seems that these materials do not fulfil all the mentioned requirements for GTR procedures.

1.5.2 Resorbable barriers

Resorbable membranes do not require additional surgery, reduce patient discomfort and costs, and eliminate potential surgical complications. Resorbable barriers can be natural or synthetic. Collagen is the most commonly used natural barrier membrane. Collagen is acquired from animal skin, tendons, intestines or pericardium. Locci and co-workers (1997) compared collagen and PTFE biocompatibility and showed that PTFE inhibited gingival fibroblasts DNA synthesis, while collagen membrane stimulated proliferation of these cells. Besides, PTFE membrane significantly reduced extracellular matrix synthesis, so results stand in favour of collagen biocompatibility.[87] Wang and co-workers (2002) showed higher adherence of osteoblasts to surfaces of collagen than non-collagen membranes.[167] Meta-analysis of clinical GTR investigations showed equal effectiveness to nonresorbable.[50] The collagen membrane appears to be useful and beneficial material for regenerative therapy in the treatment of periodontal defects. Other natural products tested for GTR without success were dura matter,[61, 176] oxydized cellulose,[58, 86] and laminar bone.[148]

Synthetic resorbable materials are usually organic aliphatic thermoplastic polymers. The materials most commonly used are poly-α-hydroxy acids, which include polylactic-polyglycolic acid and their copolymers. One of the advantages of polyhydroxy acids is hydrolysis to final products water and carbon dioxide. Carbon dioxide can cause tissue irritation due to the

formation of carbonic acid thereby creating an acidic environment. Degradation time can vary, lengthened through the addition of lactides or glycols.[21, 94]

A double-layered absorbable membrane (Guidor®) made of polylactic acid and a citric acid ester acetyl tributylcitrate was the first to appear on the market. The design of Guidor is a multilayer matrix, which facilitates the ingrowth of gingival connective tissue from the inner aspect of the periodontal flap. This ingrowth is assumed to retard and prevent the apical downgrowth of gingival epithelium. Resorbable barrier provided the advantage of eliminating the second surgery to retrieve the undegraded barrier membrane. This second surgery may disrupt initial healing and limit the overall attachment gain. The use of the membrane in single site recession eliminated the problems associated with conventional grafting which includes colour and tissue texture alteration and patient discomfort due donor site on the palate. In a survey by Roccuzzo in 1996, all patients preferred the Guidor treatment for better comfort.[139] Patients clearly preferred the single site GTR technique since they can avoid the palatal wound.

Gottlow et al in 1994 evaluated the use of resorbable barrier in recession type and interproximal defects in nonhuman primates. Clinical healing following surgery progressed with minimal or no gingival inflammation. Histological evaluation demonstrated the new cementum with inserting periodontal ligament fibres extending to the coronal border of barrier together with new bone formation. After 6 months, the barrier was completely resorbed.[65]

The following years witnessed the publications of further research works by Polson et al and Genon et al. Polson and co-workers were involved in the multicentered study of Guided Tissue Regeneration in human furcation defects after using a biodegradable barrier. A total of 29

patients with class II furcation defects were treated using polylactic acid biodegradable barrier. At twelve month post surgery, there was clinically and statistically significant improvement in mean pocket depth reduction (2.2 mm) and attachment level vertical gain (1.7 mm), and attachment level horizontal gain (2.5 mm). These results indicated favourable clinical regenerative outcomes after using this barrier material in class II furcation defects in humans.[127]

Genon et al presented data from 16 cases in which the Guidor matrix barrier was used in conjunction with the coronally position flap to treat the recession defect. Gingival recession was reduced on average by 3.7mm with gingiva up to or within 1mm of cemento-enamel junction in 9 of 16 patients. Clinical attachment level improved; a mean attachment gain of 3.9 mm was attained.[62]

The use of polyurethane for membrane production has been tested as wel.[58, 151, 169] Polyurethanes are organic polymers containing urethane group -NH-CO-O-, materials with diverse properties. Polyether urethanes are degraded through enzymatic and oxidative degradation.[124, 131] The degradation process although is extremely slow.

Black et al in 1994 compared the clinical response of Biomend collagen and ePTFE membranes in the treatment of class II furcation defects in 13 patients. Six months post treatment, the mean vertical probing depth reduction was 1.4 mm for the collagen barrier sites versus 1.1 mm for the nonresorbable barrier sites. The decrease of horizontal probing depth was 1.5mm and 0.8 mm for the resorbable and nonresorbable barrier treated sites, respectively. The author reported that the resorbable collagen barrier was found to be equivalent to the nonresorbable barrier in the clinical resolution of class II furcation defects.[23]

In conclusion, the principle of GTR lies in the establishment of the cells of periodontal ligament to selectively repopulate the root surface. Clot establishment and stabilization, site selection, epithelial cell exclusion, space provision, neovascularisation, and complete gingival coverage are favourable characteristics in any GTR procedure. In the future, GTR can be combined with the use of biological growth factors that allowed for selectively control the type of cells proliferated from the fibroblast precursor.

1.6 Bone grafting and the types of bone graft materials

The combination of graft materials with guided tissue regeneration is a proven modality of therapy for the treatment of intrabony defects and Class 2 furcation invasions. The use of bone grafts for reconstructing intra-osseous defects produced by periodontal disease dates back to Hegedus in 1923.[73] It was then revived in 1965 by Nabers and O'Leary.[110] Now, with the introduction of advanced bone grafting techniques and the use of sophisticated bone replacement graft materials, it is possible to increase the volume, width, and height of bone in deficient areas to regenerate the tissues supporting affected teeth and also to permit the placement of implants in their ideal positions and angulations.

A bone graft can aid in bone regeneration by three different methods, which include (i) osteogenesis, (ii) osteoconduction, and (iii) osteoinduction. Osteogenesis is the formation of new bone by the cells contained within the graft material. Osteoinduction is a chemical process in which molecules contained within the graft (bone morphogenetic proteins) convert the patient's cells into cells that are capable of forming bone. Osteoconduction is a physical effect by which the matrix of the graft forms a scaffold on which cells in the recipient site are able to form new bone.[111]

1.6.1 Classification

Bone replacement grafts can be broadly classified into human bone and bone substitutes. This can be further classified into autografts, allografts, xenografts, and alloplasts.[111]

I) Human bone

Autografts or autogenous grafts

    - Extraoral

    - Intraoral

Allografts or allogenic grafts

    - Fresh frozen bone

    - Freeze-dried bone allografts (FDBA)

    - Demineralized freeze-dried bone allografts (DFDBA)

II) Bone substitutes

 Xenografts or xenogenic grafts

    - Bovine-derived hydroxyapatite

    - Coralline calcium carbonate

Alloplasts or alloplastic grafts

    - Absorbable

    - Nonabsorbable

Historically, autografts were the first bone replacement grafts to be reported for periodontal applications. Allogenic freeze-dried bone was introduced to periodontics in the early 1970's, while demineralized allogenic freeze-dried bone gained wider application in the late 1980's. The introduction of xenografts and alloplasts for periodontal use occurred during the same time.[111]

1.6.1.1 Autografts

Autogenous grafts are harvested from the patient, from intraoral sites (such as the maxillary tuberosity of a healing extraction site) and extraoral sites (such as the iliac crests, ribs, cranium and tibial metaphyses).[59, 150] The decision to use autogenous grafts necessitates consideration of the donor site, procurement technique and handling or processing of the harvested material. Autogenous bone can be harvested intraorally, with or without processing, to yield graft materials of different forms, including cortical chips, osseous coagulum and bone blend. Many investigators have reported on the clinically successful use of intraoral autogenous grafts in the treatment of intrabony defects.[30, 81, 103] Regardless of the intraoral donor site, autografts yield regenerative responses superior to that of surgical debridement alone. Extraoral autografts such as those obtained from iliac crests have demonstrated great potential for supporting new bone growth, including clinical and histological evidence of crestal bone apposition and periodontal ligament formation. Schallhorn & Hiatt considered the fill of crestal facial and furcation defects to be more clinically predictable using iliac autografts than with intraoral cancellous bone.[150]

Autogenous grafts are non-immunogenic and contain osteoblasts and osteoprogenitor stem cells, which are capable of proliferating. These grafts, therefore, are osteoinductive. There are limitations to obtaining autogenous grafts, however, such as insufficient oral sites, the requirement for a second surgical site and morbidity at the donor site.[140]

1.6.1.2 Allografts

Allografts, bone grafts that are harvested from one person for transplantation in another, are used widely. There are three main divisions: frozen, freeze-dried and freeze-dried demineralized.

The possibility of disease transfer, antigenicity and the need for extensive cross-matching has disallowed the use of fresh frozen bone in modern periodontics. The evidence that freeze-drying markedly reduces the antigenicity and other health risks associated with fresh frozen bone, as well as the favourable results obtained in the field trials with freeze-dried bone allografts, have led to the extensive use of freeze-dried bone allografts in the treatment of periodontal osseous defects.[54, 105] The use of cortical bone is recommended rather than cancellous bone allografts since cancellous bone is more antigenic and there is more bone matrix and consequently more osteoinductive components in cortical bone. Freeze-dried bone allograft is regarded as osteoconductive.[64] The blockade of the effect of bone growth stimulating factors sequestered in bone matrix, like the bone morphogenic proteins, led to the development of demineralised allografts. Experimental animal studies have shown that demineralised freeze-dried bone allograft has osteogenic potential.[101, 102]

The advantages of using allografts are that the material is available in large quantities and there is no donor site within the patient. The disadvantages are that the process for preparing the graft (that is, freeze-drying and irradiating) decreases the material's integrity and osteogenic potential, and the immunological response to it may diminish its incorporation into the recipient bone. A major concern with allografts in general is the potential for disease transfer, particularly viral transmission and more particularly HIV.[104] Also, there is a need for extensive cross-matching to decrease the likelihood of both graft rejection and disease transmission.

1.6.1.3 Xenografts

Xenografts are made of naturally derived deproteinised cancellous bone from another species (such as bovine or porcine bone). The risk of transmission of diseases such as bovine spongiform

encephalopathy is negligible because the bone's organic component is extracted. After the extraction of the organic components, the remaining inorganic structure provides a natural architectural matrix as well as an excellent source of calcium. The inorganic material also maintains the physical dimension of the augmentation during the remodelling phases. Bovine-derived hydroxyapatite bone replacement grafts increase the available surface area that can act as an osteoconductive scaffold due to their porosity and have a mineral content comparable to that of human bone, allowing then to integrate with the host bone. These grafts are prepared by chemical or low-heat extraction of the organic component from the bovine bone. Examples of commercially available bovine-derived bone replacement grafts are Bio-Oss[®] (Osteohealth Co., Shirley, NY) and Osteograf/N[®] (CeraMed Dental, LLC, Lakewood, CO).

Coralline calcium carbonate graft is obtained from a natural coral, genus *Porites.* It is hugely porous similar to that of spongy bone and so it provides a large surface area for resorption and replacement by bone.[69, 175] An example for such type of grafts is Biocoral[®] (Inoteb, Saint Gonnery, France). Biocoral has a high osteoconductive potential because no fibrous encapsulation has been reported.

The main advantages of xenografts are that they are osteoconductive and readily available. A major disadvantage of bovine-derived grafts is due to the fact that it can cause disease transmission, which was evident in the case of bovine spongiform encephalopathy reported in Great Britain.[111]

1.6.1.4 Alloplasts

The alloplastic grafts or synthetic bone graft substitutes as yet offer only a part solution to the management of localized bone loss. They possess some of the desired mechanical qualities of bone as well as osteoconductive properties but are largely reliant on viable periosteum/bone for their success. They primarily function as defect fillers. Ideally synthetic bone graft substitutes should be biocompatible, show minimal fibrotic reaction, undergo remodelling, should have a similar strength and elasticity to that of the bone being replaced, thereby supporting the new bone formation. They do not induce adverse local tissue reaction, immunogenicity or systemic toxicity. They can be classified, by their ability to be bioabsorbed, into absorbable and non-absorbable.

The absorbable materials include alpha and beta tricalcium phosphate, non-sintered hydroxyapatite, and calcium sulphate. The non-absorbable materials include sintered hydroxyapatite, bioglass and HTR[TM] polymer.

Bioceramic alloplasts are comprised mainly of calcium phosphate, with the proportion of calcium and phosphate similar to bone. The two most widely used forms are tricalcium phosphate and hydroxyapatite.

Tricalcium phosphate is a porous form of calcium phosphate. Alpha and beta tricalcium phosphate are produced similarly, although they display different resorption properties. The crystal structure of alpha tricalcium phosphate is monoclinic and consists of columns of cations while the beta tricalcium phosphate has a rhombohedral structure. The former is formed by heating the later above 1180 °C and quenching in air to retain its structure.[63] Alpha form is less

stable than beta and forms the stiffer material calcium-deficient hydroxyapatite when mixed with water.[160] The most commonly used form is beta tricalcium phosphate. It is one of the earliest calcium compounds to be used as a bone graft substitute. Structurally porous beta tricalcium phosphate has a compressive strength and tensile strength similar to that of cancellous bone. It undergoes resorption over a 6-18 month period. Unfortunately, the replacement of beta tricalcium phosphate by bone does not occur in an equitable way. That is, there is always less bone volume produced than the volume of the graft material resorbed. For this reason, the clinical use of beta tricalcium phosphate has been rather as an adjunctive with other less resorbable bone graft substitutes or as an expander for autogenous bone graft. The examples of commercially available beta tricalcium phosphate graft material are Synthograft[TM] (Bicon, Boston MA, USA) and Cerasorb[®] (Curasan Pharma GmbH, Kleinostheim, Germany).

The next calcium phosphate preparation to become available was the synthetic hydroxyapatite in the 1970's. It is available in resorbable and non-resorbable forms. Whether synthetic hydroxyapatite is resorbable or non-resorbable depends on the temperature at which it is prepared. High-temperature preparation (sintering) of hydroxyapatite results in a nonresorbable, nonporous, dense material.[82] Dense non-resorbable hydroxyapatite grafts are osteophilic, osteoconductive and act primarily as inert biocompatible bone defect fillers. Histologically, new attachment is not achieved but yield a more stable clinical improvement than with open flap debridement alone in the treatment of periodontal osseous defects.[57, 174, 177] The resorbable form is processed at a low temperature. As it resorbs, a readily available source of calcium becomes available in sites that have osteogenic potential.[136] Its reported advantage is the slow resorption

rate, allowing it to act as a mineral reservoir at the same time acting as a scaffold for bone replacement.[166] It is marketed in different trade names like Osteogen® (Impladent, NY, USA).

Calcium sulphate or plaster of Paris was first documented as being used for fracture treatment by the Arabs in the 10th century, who would surround the affected limb in a tub of plaster. In 1852 a Dutch army surgeon named Mathysen incorporated plaster into a bandageable form, which we are familiar today.[106] Calcium sulphate is thought to act as an osteoconductive matrix for the ingrowth of blood vessels and associated fibrogenic and osteogenic cells. For this to occur it is critically important that the implanted calcium sulphate is adjacent to viable periosteum or endosteum.[36] Over a period of 5–7 weeks, calcium sulphate is reabsorbed by a process of dissolution.[20] Currently, a medical grade of calcium sulphate impregnated with tobramycin is commercially available (Osteoset®; Wright Medical Technology, Arlington, TN, USA). Calcium sulphate in its set form has a compressive strength greater than cancellous bone and a tensile strength slightly less than cancellous bone. Calcium sulphate, however, requires a dry environment to set and if it is re-exposed to moisture it tends to soften and fragment. For this reason it has no reliable mechanical properties *in vivo* and its application should be limited to a contained area. Hence the primary use of calcium sulphates should be as bone void filler. Bioactive glass is a silicone-based, osteoconductive material that bonds to bone through the formation of carbonated hydroxyapatite. When exposed to tissue fluids, bioactive glasses are covered by a double layer composed of silica gel and a calcium-phosphorous rich (apatite) layer. The later promotes adsorption and concentration of proteins utilized by osteoblasts to form a mineralized extracellular matrix. It has been believed that these bioactive properties guide and promote osteogenesis, allowing rapid formation of bone. Examples of bioactive glasses

commercially available are Perioglas® (Block Drug Co., NJ, USA) and Biogran® (Orthovita, PA, USA).

HTR™ synthetic bone (Bioplant, CT, USA) is a biocompatible microporous composite of methylmethacrylate and hydroxymethylmethacrylate polymers and calcium hydroxide. HTR stands for hard tissue replacement. Its hydrophilicity enhances clotting, and its negative particle surface charge allows adherence to bone. It appears to serve as a scaffold for bone formation when in close contact with alveolar bone. Histological evidence of new bone formation on HTR™ particles has been reported.

Alloplasts can be mixed with autogenous grafts or allografts in the management of large structural defects. Some alloplastic materials are mixed together to achieve superior results. Fortoss® Vital (Biocomposites, Staffordshire, UK) is such a mixture of beta tricalcium phosphate and calcium sulphate. This can be used for guided tissue regeneration without an additional membrane as calcium sulphate serves the purpose of a membrane.

To conclude, bone grafting is now a well-recognized choice in the treatment of periodontal osseous defects, especially when used along with barrier membranes. Various types of bone grafts and also their combinations are used with varying degrees of success. Rapid developments in this particular field are leading us towards achieving the ultimate goal in periodontal therapy, which is the regeneration of lost periodontal tissues. Although complete regeneration is now a distant dream, the use of bone grafts enabled us to inch towards it. Autografts are still considered the ideal grafts but for the difficulty in obtaining it. So with the source limitations of autogenous bone and concerns regarding allogenic bone, the role of bone substitutes will likely to increase.

Future of bone grafts is likely to lie in the industrially manufactured biomaterials in combination with laboratory-grown cells developed by tissue-engineering.

2 AIM OF THE STUDY

The study was aimed towards the long-term clinical evaluation of the effectiveness of a composite material, beta tricalcium phosphate in combination with calcium sulphate, in the treatment of periodontal osseous defects. Intra-bony defects remain a significant therapeutic problem in periodontal therapy. Regeneration of lost periodontal tissues is the ideal goal in the treatment of periodontal defects. Bone grafts are used mainly for the filling of the bony defects thereby aiding in regeneration. The indications of various bone grafts in periodontal therapy are similar, but the search for the ideal material is still on. This study was focused on one such synthetic graft material which could be superior to other graft materials in terms of clinical outcome and usage owing to its properties.

3 OUTLINE OF THE STUDY

This long-term retrospective follow-up study was designed to evaluate the clinical outcome of guided tissue regeneration with a synthetic bone graft material.

Forty-seven intrabony defects in twenty-six periodontitis patients were treated with the bone replacement composite graft material beta tricalcium phosphate in combination with calcium sulphate. Patients were recalled for the postoperative care at two weeks, three months, six months, one year and two years.

Clinical parameters were evaluated which included changes in probing depth (PD), clinical attachment level/loss (CAL) and gingival recession (GR) at the baseline (preoperative), at one-year and two-years postoperatively along with the various factors like presence of plaque, bleeding on probing (BOP) and smoking.

The preoperative measurements were compared to the postoperative measurements at one and two years to determine whether the technique had a statistically significant effect on the outcome of the treatment. Other factors like sex, smoking, oral hygiene were also evaluated to determine whether they could be related to improved or diminished results. Radiographs were made preoperatively and at one and two years postoperatively.

4 MATERIALS AND METHODS

4.1 Subjects

In this study twenty six patients who were consecutively treated using the composite bone replacement graft material were evaluated. These patients had moderate to advanced chronic periodontitis, were in general good health presented with at least one deep intrabony defect and were treated in the Division of Periodontology, Department of Dentistry, Faculty of Medicine in Hradec Králové, Czech Republic. They were aged 21 to 58 years with a mean age of 42.27±10.66 at the time of surgery. There were 9 males and 17 females (Fig. 4.1), out of which 8 were smokers (Fig. 4.2). All the smokers were medium smokers, smoking up to 5 cigarettes a day.
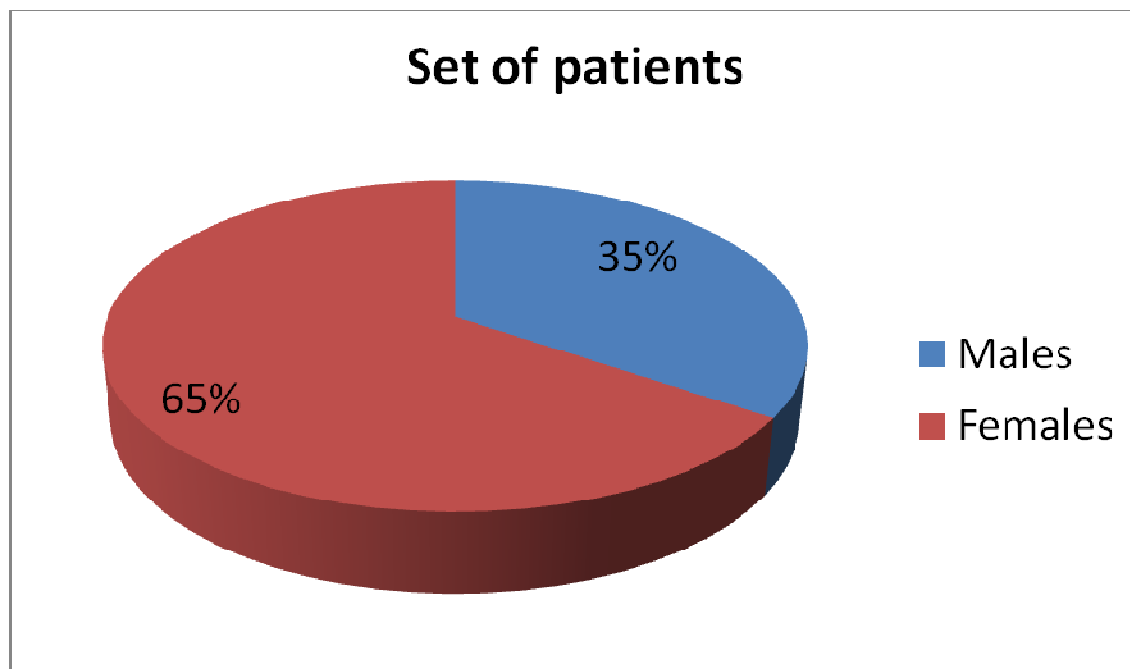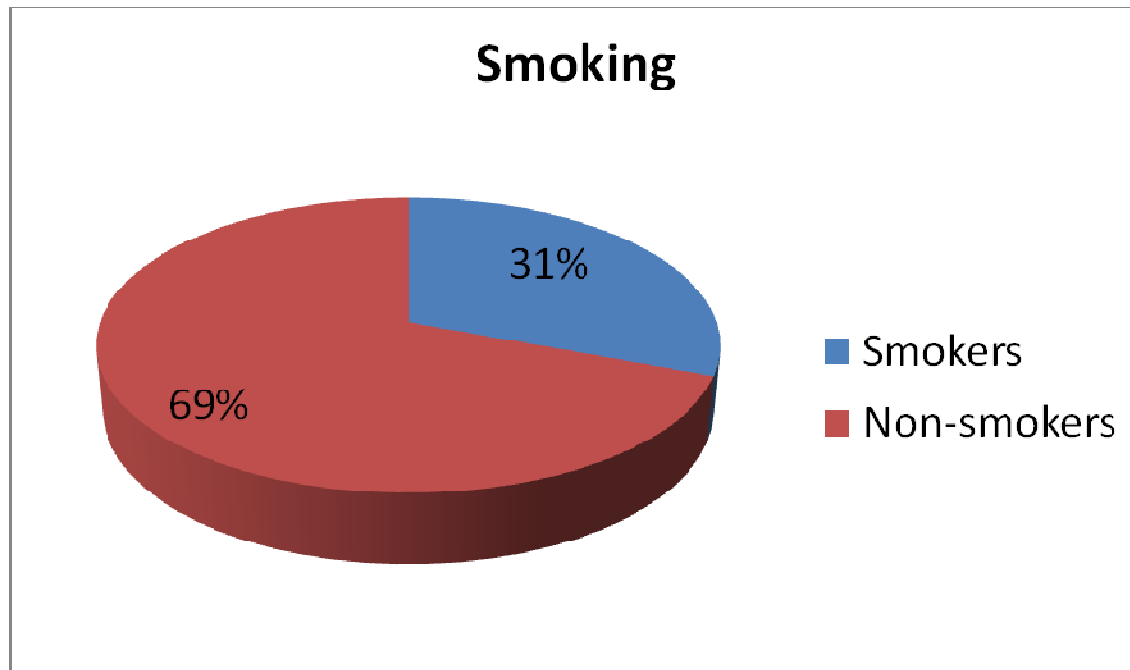
Figure 4.1

Figure 4.2

Subject inclusion was based on the presence of at least one tooth with a probing depth (PD) of $\geq$ 5 mm and radiographic evidence of intrabony defect after initial phase of periodontal therapy. The exclusion criteria consisted of patients with systemic diseases or medically compromised conditions and taking any drug known to interfere with the wound healing during the previous six months, pregnant and/or lactating women and insufficient dental hygiene characterized by a papilla bleeding index (PBI) total score of >15. Teeth had to be vital or properly treated with root canal therapy. Signed consent forms before surgeries were obtained from the patients.

Each patient received an initial periodontal treatment including oral hygiene instructions, plaque control and full mouth scaling and root planning. Persistent deep periodontal pockets (PD $\geq$ 5 mm) with bleeding on probing (BOP) after the initial phase therapy and the maintenance phase varying between two to four months were considered for the surgical treatment with the bone

graft material. Radiographic assessment of the sites provided further evidence of intrabony defects. A total of forty seven teeth (33 frontals + 7 premolars + 7 molars) with intrabony defects which were two or three-walled in twenty six patients were treated in a period of about two years at our clinic (Fig. 4.3).

Figure 4.3
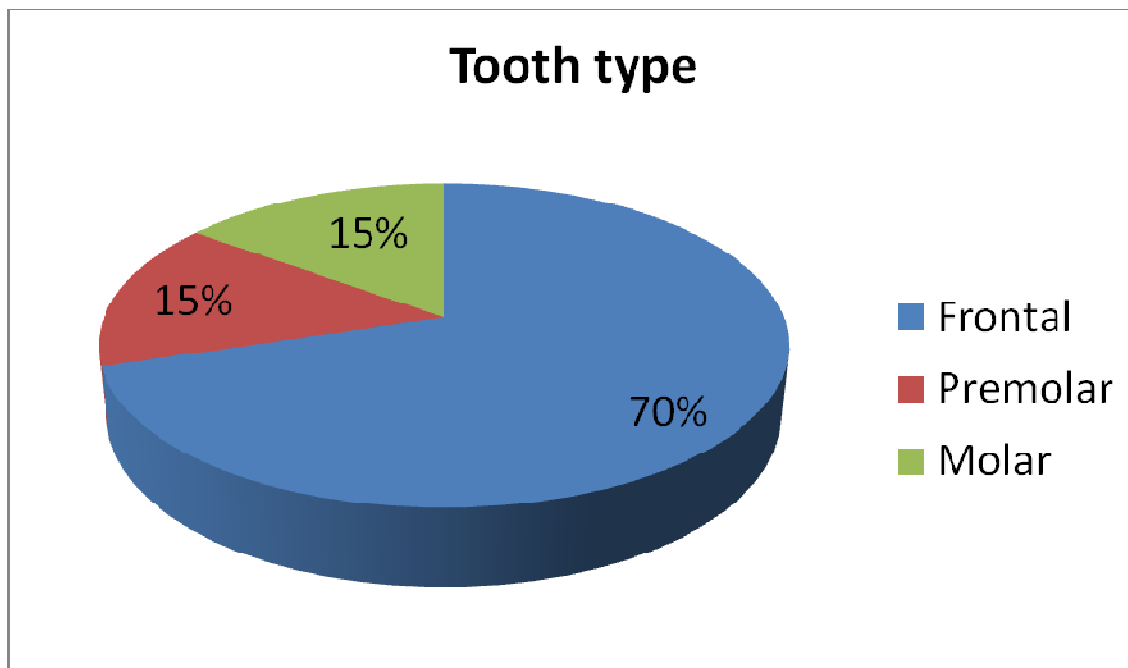
4.2 Materials

4.2.1 Fortoss® Vital

Fortoss® Vital (Biocomposites, Staffordshire, UK) which is a comparatively newer biphasic calcium composite material composed of a porous beta tricalcium phosphate and calcium sulphate is being used in the treatment of periodontal intrabony defects in our dental clinic since

the year 2003. Due to a modified surface activity and ion loading, its osteoconductive behaviour seems to be superior if compared to conventional calcium phosphates. This material has the benefit of being both a graft material and an integral membrane produced within one mixture. The beta tricalcium phosphate acts as a slowly resorbing matrix that is substituted by bone. The calcium sulphate sets hard and acts as a resorbable membrane that stabilises the graft but excludes any competitive cells. It also exhibits Zeta Potential Control (ZPC™, Biocomposites, Staffordshire, UK) which is claimed to enhance bone growth by attracting bone proteins into the site from the surrounding tissues. Fortoss® Vital is supplied as a sterile, sealed and disposable kit. The kit consists of a vial containing the graft powder, a solution container, a pipette, a mixing bowl and a spatula (Fig. 4.4).

Figure 4.4
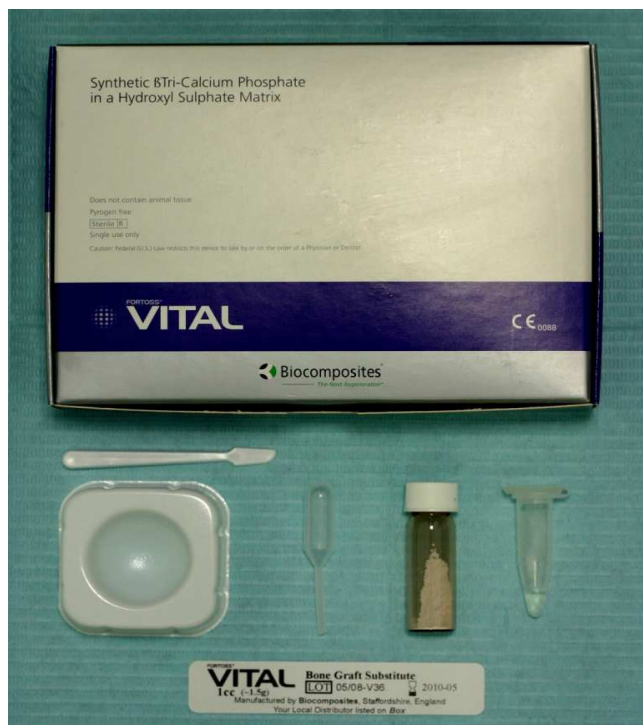
4.2.2 Tetracycline hydrochloride

Tetracycline hydrochloride solution was prepared by slowly adding tetracycline hydrochloride powder into distilled water until a saturated solution of approximately 50 mg/ml concentration was obtained with constant stirring (Fig. 4.5). Topical tetracycline HCL conditioning removes the smear layer and is believed to enhance fibroblast attachment and growth, while suppressing epithelial cell attachment and growth and also has an anticollagenase action. Furthermore, topical tetracycline HCl is adsorbed to and released from the dentin surface maintaining an antimicrobial property for at least fourteen days post therapy.

Figure 4.5

4.3 Methods

4.3.1 Pre-surgical phase

The pre-surgical phase consisted of proper evaluation and selection of periodontal intrabony defects for surgery following initial phase periodontal therapy. Initial phase of treatment consisted of controlling the etiological factors, thereby halting the further progression of the disease. This phase, also called as etiologic or hygienic phase, included patient motivation and education in matters of oral hygiene, elimination of supragingival and subgingival dental calculus and contaminated radicular cementum and modification/elimination of other plaque retentive features like restoration overhangs. Teeth with hopeless prognoses were duly extracted. The standard procedure employed for elimination of subgingival calculus and other unwanted contents of the periodontal pocket was scaling and root planing (SRP) using a set of Gracey's curettes (Hu-Friedy, Chicago, IL, USA) (Fig. 4.6). Once the subgingival treatment was completed, patients were recalled after 6-8 weeks for follow-up. In the follow-up examination, oral hygiene was assessed and periodontal probing depths were measured again. To ensure the uniformity in the probe diameter, a Williams probe (Hu-Friedy, Chicago, IL, USA) was used throughout the study (Fig. 4.7). The surgical treatment phase was initiated after completion of the initial phase of periodontal therapy and scheduled recall. The sites for surgical treatment were selected, during the recall phase, according to the presence of deep periodontal pockets with bleeding on probing (BOP) with radiographic evidence of intrabony defects.
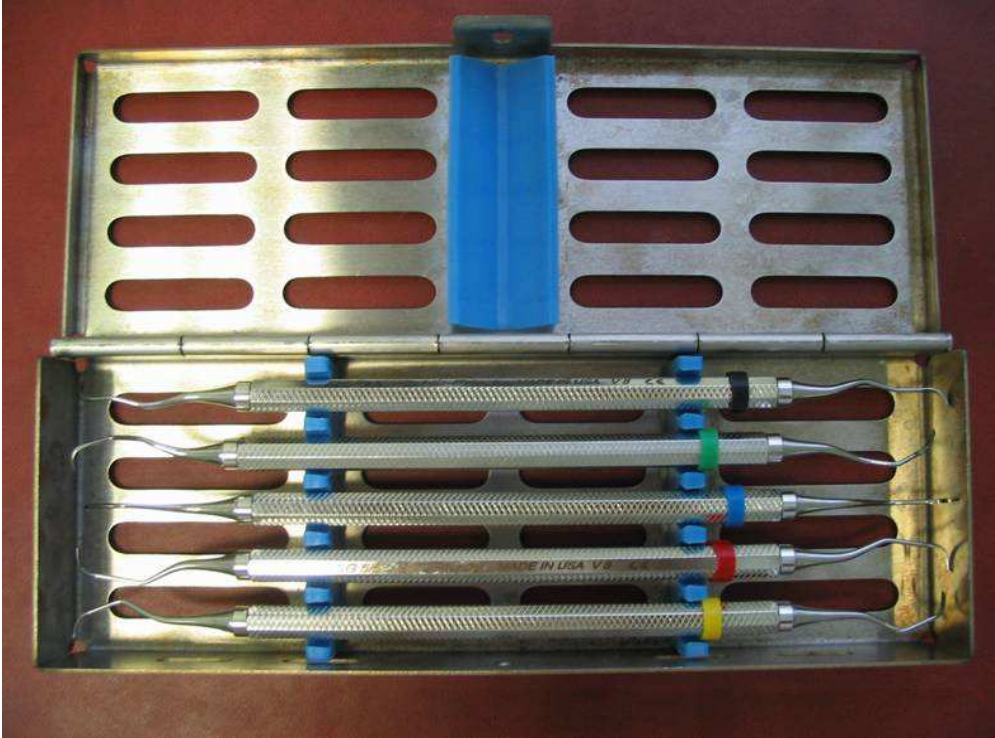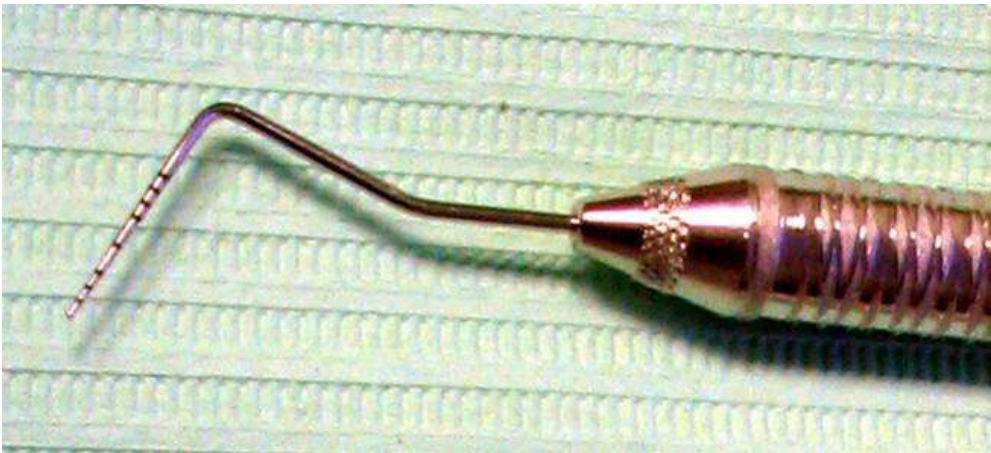
Figure 4.6

Figure 4.7

4.3.2 Surgical phase

Surgical phase was initiated only if the patients were presented with adequate oral hygiene. Signed informed consents were obtained from all patients before surgery.

All the patients were treated under local anaesthesia (Articaine hydrochloride 4% with 1: 120,000 epinephrine hydrochloride). After achieving sufficient local anaesthesia, full-thickness mucoperiosteal flaps were elevated using a crevicular incision on the facial and lingual surfaces of each tooth, segment or area involved. In the upper anterior regions papilla preservation incisions were made in the interdental area (Fig. 4.8). Vertical release incisions were used as necessary. Surgical scalpels number 11, 15 and a micro scalpel number 67 (Fig. 4.9). After the elevation of the flap (Fig. 4.10) using periodontal periosteal elevators (Fig. 4.11), a thorough root surface debridement was done using Gracey or universal curettes. All granulomatous tissue were removed from the osseous defects and rinsed with saline (Fig. 4.12). Root surface conditioning was done using 2.5% tetracycline hydrochloride for 2-3 minutes followed by flushing with saline. Fortoss® Vital powder is mixed with the fluid supplied along with it in to a gritty mouldable paste and applied it in layers using a sterile instrument (Fig. 4.13, 4.14). The graft material was firmly pressed into the site using finger pressure over sterile gauze. The defects were over-packed to allow for any settling of the mixture (Fig. 4.15). Any excessive blood was removed from the site by using damp sterile gauze. Then the gauze was held on the graft for a few seconds. The mucoperiosteal flaps were approximated and sutured (Fig. 4. 16). The sutures used were resorbable sutures (Safil®, Braun, Tuttlingen, Germany) (Fig. 4.17).
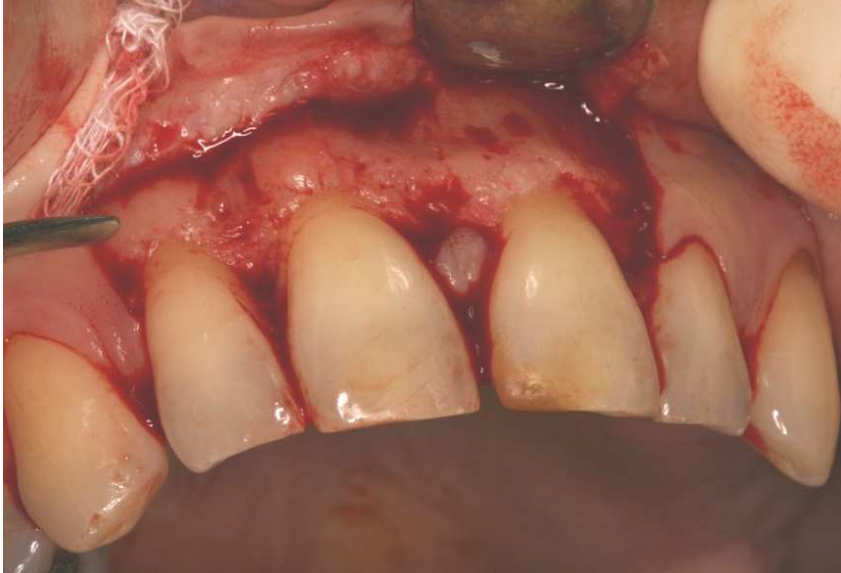
Figure 4.8

Crevicular and papilla preservation incision (arrows) along with vertical incisions made to expose the intrabony defect related to the upper left central incisor in a patient.

Figure 4.9

Figure 4.10

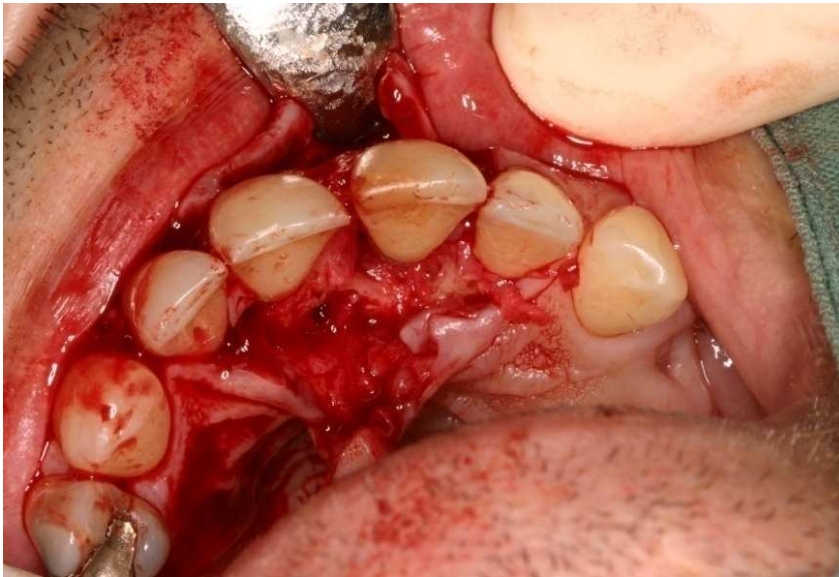Full-thickness mucoperiosteal flap elevated

Figure 4.11

Figure 4 .12

Defect mechanically debrided and rinsed with saline.

Figure 4. 13

Fortoss ® Vital powder and fluid with dropper as supplied by the manufacturer.

Figure 4.14

Powder mixed with fluid in to a gritty mouldable paste
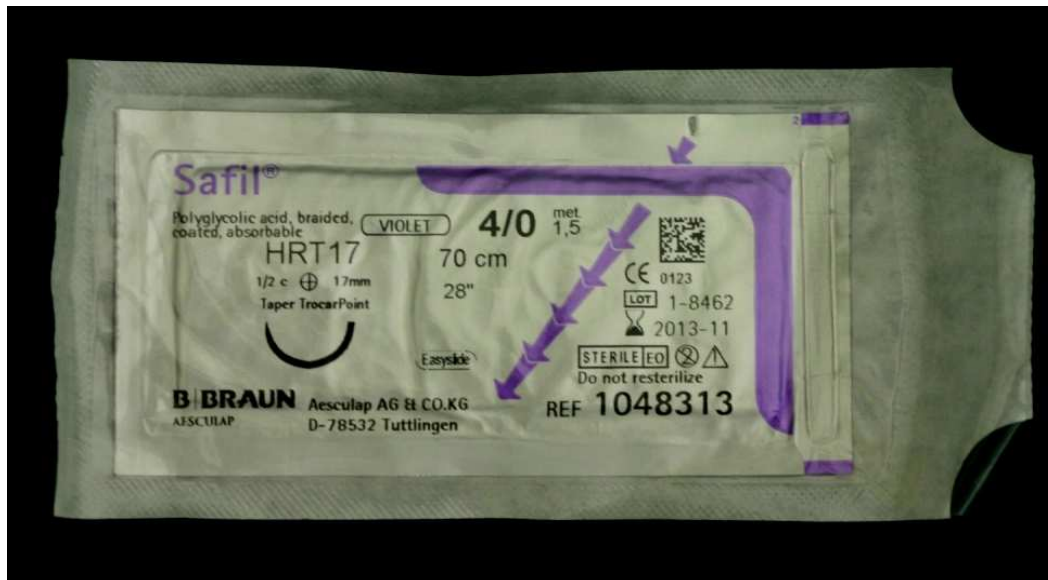

Figure 4.15

Graft material applied in to the bony defect

Figure 4.16

Sutures placed

Figure 4. 17

4.3.3 Post-surgery

The patients were given post-operative instructions including rinsing with Listerine® (Johnson & Johnson, Maidenhead, UK) mouth rinse for two weeks. Antibiotics (Amoxicillin 250 mg with clavulanic acid 125 mg or clarithromycin 250 mg) were prescribed post-operatively as surgical prophylaxis to the patients for 7 - 14 days. The sutures were removed after two weeks and the surgical sites were cleansed gently with 3% hydrogen peroxide using a cotton swab. The patients were scheduled for recall visits at 3, 6, 12 and 24 months postoperatively. Oral hygiene was evaluated and supragingival prophylaxis was carried out at each recall visit. Clinical parameters were also measured during the recall visits.

4.3.4 Clinical measurements

The clinical measurements were performed by two examiners randomly. Both the examiners recorded similar measurements during a two year trial period of cross-checking. Clinical parameters were recorded just before the surgery (baseline) and at one and two years postoperatively. These included probing depth (PD), gingival recession (GR), clinical attachment level/loss (CAL), presence/absence of dental plaque on the mesial and distal tooth surfaces, and bleeding on probing (BOP). The measurements were done using a calibrated periodontal probe (Williams colour coded, Hu-Friedy, Chicago, IL, USA) at the buccal/vestibular, lingual/oral, mesial and distal surfaces on all teeth involved and the highest value for each surface was quoted.

4.3.5 Radiographs

Intraoral periapical radiographs were taken at the baseline and at 2 years postoperatively. The radiographs were used only for the detection of bone fill in the defects and not for the measurements as the method employed were not standardized. Post-operative radiographs were compared to the ones at the baseline in order to evaluate the bone fill and also to compare that with the clinical measurements.

4.3.6 Statistical methods

Comparisons between baseline and one year and that between baseline and two year data were made using paired t-tests and Fisher's exact tests. Mean differences in the PD, GR and CAL were calculated on individual surfaces separately as well as together. The changes in other variables like BOP and plaque deposits were also evaluated. All the surfaces of an involved tooth were taken into account irrespective of the presence/absence of PD $\geq$ 5mm. This was done to assess the outcome of surgery on the non-involved sites of the involved tooth as well. Data were expressed as means $\pm$ standard deviation. The level of significance was set at 0.05.

5 RESULTS

Clinically, the graft material used was easy to handle, strongly adherent, packed well into defects, appeared to harden as a solid in a few minutes and biocompatible. Wound healing was uneventful. No patients reported a significant postoperative pain during the first week. 8 patients did not turn up for all scheduled recall visits. All of them reported at 2 years postoperatively.

A decrease in probing depths (PD) was noticed in 24 patients out of the total 26 at one year postoperatively. At 2 years postoperatively, a decrease in PDs was found in all patients but one. The number of BOP positive sites in relation to the involved teeth was reduced from 67 (35.64 %) at baseline to 26 (13.83 %) at 1 year and 28 (14.89 %) at 2 years postoperatively. The number of sites with presence of plaque got decreased from 25 (26.60 %) to 15 (15.96 %) and then increased slightly to 18 (19.15 %) during the same interval. The difference between the percentage of plaque deposits at the baseline and 1 year and between baseline and 2 years were statistically significant as shown in Table 5.1.

Table 5.1

Plaque and bleeding on probing (BOP) sites at the baseline and at 1 and 2 years postoperatively

Parameter	Baseline	1 year	2 years	*P*
Plaque	35. 64 %	13.83 %	14.89 %	0.0001
BOP	26.60 %	15.96 %	19.15 %	0.0001

The mean PD was 4.07 ± 2.63 mm at the baseline and 2.10 ± 1.29 mm at 1 year postoperatively and 2.0 ± 1.2 mm at 2 years postoperatively. When only the deepest probing measurements (≥ 5 mm) corresponding to deep intrabony defects were considered, the mean PD was 6.67 ± 1.49 mm at the baseline and 2.67 ± 1.35 mm at 1 year and 2.60 ± 1.46 mm at 2 years postoperatively (Tab. 5.2).

Table 5.2

Probing depth (PD) measurements (mean values) at baseline, 1 year and 2 years postoperatively

Mean PD (mm)	Baseline	1 year	2 year
Total measurements around involved tooth	4.07 ± 2.63	2.10 ± 1.29	2.0 ± 1.2
Single deepest measurement representing intrabony defect	6.67 ± 1.49	2.67 ± 1.35	2.60 ± 1.46

Similarly, the mean CAL was 5.66 ± 3.10 mm at the baseline and 3.94 ± 1.96 mm at 1 year postoperatively and 3.73 ± 1.90 mm at 2 years postoperatively. When only the deep probing measurements corresponding to deep intrabony defects were considered, the mean CAL was 7.66 ± 2.29 mm at the baseline and 4.72 ± 1.87 mm at 1 year and 4.43 ± 1.95 mm at 2 years postoperatively (Tab. 5.3).

Table 5.3

Clinical attachment level (CAL) measurements (mean values) at baseline, 1 year and 2 years postoperatively

Mean CAL (mm)	Baseline	1 year	2 year
Total measurements	5.66 ± 3.10	3.94 ± 1.96	3.73 ± 1.90
Single deepest measurement	7.66 ± 2.29	4.72 ± 1.87	4.43 ± 1.95

The changes in parameters illustrated in the tables 5.2 and 5.3 between baseline and 1 year postoperatively and between baseline and 2 years postoperatively were statistically significant with p values less than 0.0001. The differences in parameters between 1 and 2 years postoperatively were not found to be statistically significant with p values greater than 0.05.

Tables 5.4, 5.5 and 5.6.A and B show the differences in deep probing depth measurements at the baseline, at 1 year and at 2 years postoperatively. There were a total of 83 PD measurements in those 47 involved teeth which were ≥ 5 mm at the baseline. A 1 year postoperative recall check showed a significant decrease in this number to 14. At 2 years postoperatively, this number was found to be 13. There was a significant decrease in the numbers in relation to the frontal teeth, but a perfect outcome was resulted in case of these numbers in molars and also in the vestibular aspect of all teeth involved.

Table 5.4

PD measurements ≥ 5 mm at baseline

Teeth	V	M	O	D
Frontal	8	27	11	11
Premolar	0	4	3	4
Molar	1	6	3	5
Total	*9*	*37*	*17*	*20*

V = vestibular/buccal, M = mesial, O = oral/lingual, D = distal

Table 5.5

PD measurements ≥ 5 mm at 1 year postoperatively

Teeth	V	M	O	D
Frontal	0	4	4	4
Premolar	0	2	0	0
Molar	0	0	0	0
Total	*0*	*6*	*4*	*4*

Table 5.6.A

PD measurements ≥ 5 mm at 2 years postoperatively

Teeth	V	M	O	D
Frontal	0	4	3	4
Premolar	0	2	0	0
Molar	0	0	0	0
Total	*0*	*6*	*3*	*4*

Table 5.6.B

Graphic representation of distribution of probing depths ≥ 5 mm
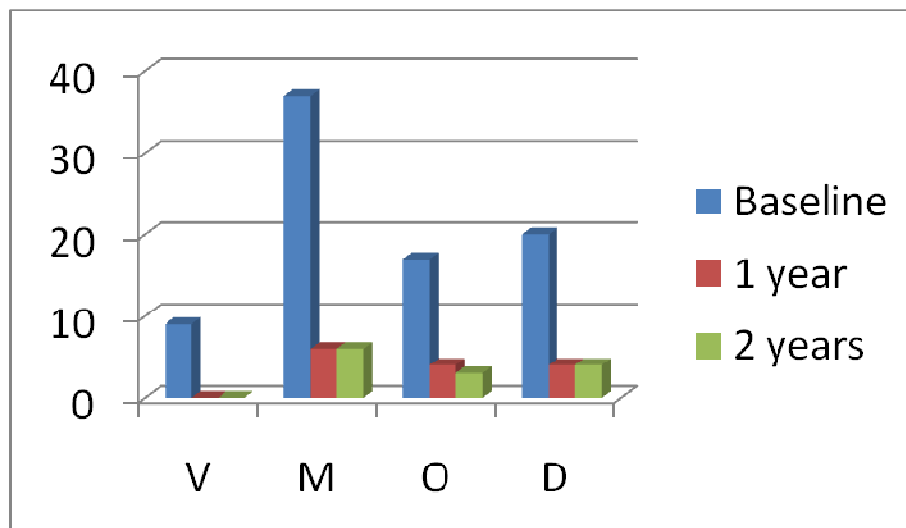
Tables 5.7, 5.8 and 5.9 show how the CAL measurements fared during the same period. Total CAL measurements of ≥ 5 m at the baseline was 105 which got reduced by almost half to 61 at 1 year and 54 at 2 years postoperatively. Again, the numbers were much more significant in relation to the frontal teeth and in the proximal and oral aspects.

Table 5.7

CAL measurements ≥ 5 mm at baseline

Teeth	V	M	O	D
Frontal	9	27	20	16
Premolar	0	4	3	6
Molar	4	7	4	5
Total	*13*	*38*	*27*	*27*

Table 5.8

CAL measurements ≥ 5 mm at 1 year postoperatively

Teeth	V	M	O	D
Frontal	4	12	7	10
Premolar	0	4	1	4
Molar	4	6	4	5
Total	*8*	*22*	*12*	*19*

Table 5.9

CAL measurements ≥ 5 mm at 2 years postoperatively

Teeth	V	M	O	D
Frontal	7	17	8	10
Premolar	0	0	1	1
Molar	3	4	1	2
Total	*10*	*21*	*10*	*13*

The mean differences in measurements between the baseline and one year postoperatively are a reduction of 1.97 ± 1.15 mm (P= 0.0001) in case of PD, a gain of 1.68 ± 1.12 mm (P = 0.0001) in CAL and an increase of 0.30 ± 0.71 mm (P = 0.009) in GR. The mean differences in measurements between the baseline and two years postoperatively are a reduction of 2.07 ± 1.14 mm (P = 0.0001) in case of PD, a gain of 1.93 ± 1.36 mm (P = 0.0001) in CAL and an increase of 0.14 ± 0.73 mm (P = 0.571) in GR. These are illustrated in tables 5.10-5.16. No significance was found statistically between the results after 1 and 2 years postoperatively (P > 0.05 in case of difference in means: CAL, PD and GR)

Table 5.10

Mean difference in gingival recession (GR) measurements at baseline and 1 year postoperatively

GR				
(Increase= "+", decrease=" –")				
V	M	O	D	*Average*
+ 0.50 (-1 to 3)	+ 0.18 (-3 to 3)	+ 0.38 (-2 to 2)	+ 0.26 (-3 to 3)	*+ 0.30*
SD: ± 0.99	SD: ± 1.18	SD: ± 0.97	SD: ± 1.51	SD: ± 0.71

(Values in millimetres, maximum and minimum values in brackets, SD- standard deviation)

Table 5.11

Mean difference in gingival recession (GR) measurements at baseline and 2 year postoperatively

GR				
(Increase= "+", decrease=" –")				
V	M	O	D	*Average*
+ 0.40 (-2 to 3)	- 0.06 (-2 to 3)	+ 0.27 (-2 to 3)	- 0.04 (-3 to 2)	*+ 0.14*
SD: ± 1.17	SD: ± 1.11	SD: ± 0.94	SD: ± 1.33	SD: ± 0.73

Table 5.12

Mean difference in clinical periodontal probing depth (PD) measurements at baseline and 1 year postoperatively

PD				
(Increase= "+", decrease=" –")				
V	M	O	D	*Average*
- 0.89 (-7 to 3)	- 3.10 (-9 to 5)	- 1.51 (-6 to 2)	- 2.38 (-9 to 1)	*- 1.97*
SD: ±1.91	SD: ±2.75	SD: ±1.99	SD: ±2.26	SD: ±1.15

Table 5.13

Mean difference in periodontal probing depth (PD) measurements at baseline and 2 year postoperatively

PD				
(Increase= "+", decrease=" –")				
V	M	O	D	*Average*
- 0.96 (-6 to 1) SD: ± 1.18	- 3.49 (-8 to 2) SD: ± 2.47	- 1.74 (-6 to 2) SD: ± 2.16	- 2.11 (-9 to 1) SD: ± 2.15	*- 2.07* SD: ± 1.14

Table 5.14

Mean difference in clinical attachment level (CAL) measurements at baseline and 1 year postoperatively

CAL				
(Gain= "+", Loss= "-")				
V	M	O	D	*Average*
+ 0.36 (-2 to 7) SD: ± 1.88	+ 2.94 (-4 to 9) SD: ± 2.89	+ 1.13 (-2 to 5) SD: ± 2.10	+ 2.10 (-3 to 8) SD: ± 2.59	*+ 1.68* SD: ± 1.12

Table 5.15

Mean difference in clinical attachment level (CAL) measurements at baseline and 2 years

postoperatively

CAL				
(Gain= "+", Loss= "-")				
V	M	O	D	*Average*
+ 0.55 (-3 to 6) SD: ± 1.95	+ 3.55 (-2 to 9) SD: ± 2.60	+ 1.48 (-3 to 6) SD: ± 2.36	+ 2.13 (-3 to 8) SD: ± 2.54	*- 1.93* SD: ± 1.36

Table 5.16

Mean differences and corresponding *p* values

Parameter (change)	Mean difference between baseline and 1 year	*p*	Mean difference between baseline and 2 years	*p*
CAL (gain)	1.68 ± 1.12 mm	0.0001	1.93 ± 1.36 mm	0.0001
PD (reduction)	1.97 ± 1.15 mm	0.0001	2.07 ± 1.14 mm	0.0001
GR (increase)	0.30 ± 0.71 mm	0.009	0.14 ± 0.73 mm	0.571

There were no significant differences between smokers and non-smokers ($p = 1.000$). But in one patient where an increase in PD and CAL were noticed 2 years after the surgery, a combination of different factors like smoking, bad oral hygiene and non-compliance with the follow-up schedule during the maintenance phase after surgery were present.

Intraoral periapical radiographs showed bone fill in the defects in patients where PD got reduced after the surgical treatment (Tab. 5.17).

Table 5.17.: Comparison of intraoral periapical radiographs at the baseline and at 2 years postoperatively

	Baseline	2 years postoperatively
1)		
2)		
3)		

6 DISCUSSIONS

Bone grafting is now a well-recognized choice in the treatment of periodontal osseous defects, especially when used along with barrier membranes. Various types of bone grafts and also their combinations are used with varying degrees of success. Autografts have been considered to be the gold standard among bone replacement grafts as they can induce osteogenesis.[56, 70, 111] However, there are some limitations for the autografts like a surgical donor site is needed and availability of graft bone is limited. The alloplastic grafts or synthetic bone graft substitutes as yet offer only a part solution to the management of localized bone loss. They possess some of the desired mechanical qualities of bone as well as osteoconductive properties but are largely reliant on viable periosteum/bone for their success. They primarily serve as defect filler. In the present study, we have evaluated the effectiveness of a novel composite alloplast in the treatment of periodontal intrabony defects.

6.1 Discussion on the graft material Fortoss® Vital

Fortoss® Vital which is a combination of beta tricalcium phosphate and calcium sulphate is being used in the treatment of periodontal intrabony defects in our department since the year 2003. The main reasons for the choice of this bone graft material over the conventional membrane and graft technique to achieve periodontal regeneration are non-requirement of a membrane, reduced surgical time, lesser cost and the ease and potential to treat periodontal intrabony defects spanning more than 2 teeth. After all, the surface characteristics of the material might promote an optimal integration of the graft material with a favourable healing outcome. According to the manufacturers the material possesses an electronegative surface charge (negative Zeta potential)

and this will make it more accessible for the attachment and proliferation of osteoblasts than surfaces with no or even positive electric charge.

The use of a composite graft containing beta tricalcium phosphate and calcium sulphate was described in only a few reports and studies.[126, 157, 163, 164] In those reports and studies it was found that the use of this particular graft provided good results. In a clinical study published in 2009 by Stein et al, it was found that the clinical benefits of a biphasic composite graft containing beta tricalcium phosphate and calcium sulphate were equivalent to that of autogenous bone spongiosa and superior to that of OFD alone. At 12 months postoperatively, the patients treated with the composite graft exhibited a mean PD reduction of 3.6 ± 0.7 mm and a mean CAL gain of 3.0 ± 0.8 mm.[157] The study done on the iliac crest of dogs by Podaropoulos et al. in 2009 revealed that the mean percentage of new bone regeneration after 4 months by histological evaluation and morphometric analysis was 49.38 %.[126] Structurally porous beta tricalcium phosphate has a compressive strength and tensile strength similar to that of cancellous bone. It undergoes resorption over a 6-18 month period. Calcium sulphate has a compressive strength greater than that of cancellous bone. It can act as a barrier membrane as well, which makes it ideal for using as an adjunct with other graft materials. It requires only 5-7 weeks for complete resorption .[20, 158]

6.2 Discussion on methods

Measurement methods for the assessment of clinical outcome variables, such as probing depths, attachment level and gingival recession, have varied between studies, particularly with regard to the use of automatic or pressure sensitive or conventional probes and the use of a stent as a reference point.[132] The key element is the consistency of the assessment throughout the study. In the present study, an occlusal stent was not fabricated; the cement enamel junction and the free

gingival margin served as the reference point. Manual probes were used to measure the variables. The ability of a probe to penetrate into a periodontal pocket is related to several factors like the probing force, diameter of the probe and the gingival tissue tone. [5, 79, 137] In our study, Williams colour-coded probe (Hu-Friedy, Chicago, IL, USA) was used throughout in order to ensure the consistency in probe diameter. The clinical measurements were performed by 2 examiners randomly. Both the examiners recorded similar measurements during a 2 year trial period of cross-checking which ensured the similarity in probing force and method. Gingival tissue consistency may be modified after the placement of graft material in to the defect, which in turn can impede penetration of the periodontal probe causing false positive results.[28]

Unlike usual studies, we have considered the unaffected sides of the tooth as well. We have done this as the surgical wound included all the sides of the tooth and surgery as such can have an effect on the unaffected sides as well. The intrabony defects in this study varied in terms of the depth and type of the defect. There were 2 and 3 walled defects. The sample size used in this study was relatively small, but it was within the range of most periodontal regenerative studies.[60] Although standardised radiographs were not made in this study, 2 years after treatment radiographic defect fill with bone-like radio opaque tissue, which was indistinguishable from native bone and therefore considered as new bone, was observed. The shortcomings of the study could be a small patient group, no standardised radiographic analysis or surgical re-entry to establish the bone fill or regeneration, the non-usage of stents during clinical measurements and also the non-involvement of a control group in which another surgical technique or material was used.

Conventional therapy is capable of controlling periodontal disease. Scaling and root planning allow reduction in pocket depth mainly by new connective tissue or epithelial attachment; with a probable gain in clinical attachment level. Periodontal literature is sated with studies showing the treatment of periodontitis by scaling and root planing results in reductions of probing depth.[9, 35, 67, 68] The decrease in probing depth is caused partly by the shrinkage of the pocket soft tissue wall manifested as recession of the gingival margin which results from a decrease in soft tissue inflammation; and partly from the gain in clinical attachment.[9, 35, 67, 68] In a thorough evidence-based review, Cobb calculated the mean probing depth reduction and gain of clinical attachment that can be achieved with root planing at sites that initially were 4 to 6 mm in depth and 7 mm or greater in depth. He reported mean pocket depth reductions of 1.29 mm and 2.16 mm, respectively, and mean gains of clinical attachment of 0.55 mm and 1.29 mm, respectively.[35]

The regenerative procedures are performed after the conventional scaling and root planing to attain further improvement of the tissues destroyed by periodontitis. These procedures can promote further bone fill, thus improving the supporting structures thereby improving the long-term prognosis of the tooth.

6.3 Discussion on results

Results from present investigation showed that the graft material used was effective in significantly improving the clinical parameters at 1 and 2 years after surgery. The overall reduction in PD and gain of attachment were found to be highly statistically significant and the mean difference in GR between the baseline and at 2 years postoperatively was negligible and not significant statistically. Ideally, a comparative study with open flap debridement and/or using a different bone graft material in treating comparable defect pairs would have been more

significant to highlight the outcome of treatment using Fortoss® Vital. The amount of PD reduction was found to be greater in the deeper defects. In some cases, this reduction was up to 9 mm. PD reduction was achieved in 25 of the total 26 patients; there was an increase of PD in one patient 2 years postoperatively. The local factors and the non-compliance of the patient probably would have resulted in the undesired result. After 2 years, the number of sites with bleeding on probing was reduced to almost half. The number of proximal sites (mesial and distal) with plaque deposits also got reduced.

Several studies were done to evaluate the effectiveness of calcium sulphate and of beta tricalcium phosphate in combination with other materials resulting in good clinical outcomes. A study by Harris in 2004 evaluating a composite bone graft (demineralised freeze-dried bone allograft, calcium sulphate, tetracycline and porous hydroxyapatite) and calcium sulphate barrier showed a mean decrease of 4.7 mm of PD, 3.7 mm of CAL and a mean increase of 1.0 mm of GR at 4-6 months postoperatively.[72] In another study by Paolantonio et al. using calcium sulphate barrier implant and barrier revealed a mean decrease of 4.4 mm of PD, 2.7 mm of CAL and a mean increase of 1.6 mm of GR at 12 months postoperatively.[119] In a study published in 2008 by Döri, at 1 year after therapy, the sites treated with platelet rich plasma+ β-TCP + GTR showed a reduction in mean PD from 9.1 ±0.6 mm to 3.3 ±0.5 mm (P<0.001) and a change in mean CAL from 10.1 ± 1.3 mm to 5.7 ±1.1 mm.[45] Most of these studies used clinical measurements along with standardized radiographs for comparison. Unlike the present study, all these studies were short-term studies and have considered only the affected area around the tooth, where the pocket depths were deeper, which may influence the results.

We have used tetracycline HCl solution for root conditioning during the surgery. Topical tetracycline HCl conditioning removes the smear layer and is believed to enhance fibroblast attachment and growth, while suppressing epithelial cell attachment and growth [8] and also has an anti-collagenase action.[159] Furthermore, topical tetracycline HCl is adsorbed to and released from the dentin surface maintaining an antimicrobial property for at least fourteen days post therapy. [156] However, root conditioning using tetracycline HCl application has not proven to be beneficial in terms of clinical significance to periodontal regeneration.[95]

7 CONCLUSIONS

Within the limitations of this retrospective study, the following conclusions were drawn:

- The treatment with a synthetic bone graft containing a combination of beta tricalcium phosphate and calcium sulphate led to a significantly favourable clinical improvement in periodontal intrabony defects two years after the surgery.

- The graft material was easy to handle, strongly adherent, packed well into defects, appeared to harden as a solid in a few minutes and biocompatible.

- There was a statistically significant difference in terms of clinical attachment level (CAL) and periodontal probing depth (PD) between the baseline and one year postoperatively and between baseline and two years postoperatively. Even though there was a slight positive difference between one and two year results clinically, the difference was not statistically significant.

- A much longer term evaluation and further studies are necessary to completely ascertain the effectiveness of this material, and a larger sample size is also recommended. Also, standardized radiographic or a surgical re-entry is recommended for confirmation of the clinical results.

8 CLINICAL IMPLICATIONS

- From this study, it became evident that treatment of periodontal intrabony defects with the new graft material Fortoss® Vital offers a method to achieve significant probing depth reduction and shallow residual pockets, which are considered important for maintaining periodontal health and improving the prognosis of treated teeth.

- Fortoss® Vital can be prescribed in the treatment of 2 or 3-walled periodontal intrabony defects.

- The ease with which this material can be manipulated and its property of being a graft material and an integral membrane in one mixture allows an easy and predictable way of guided tissue regeneration procedure.

- The main reasons for the choice of this bone graft material over the conventional membrane and graft technique to achieve periodontal regeneration are non-requirement of a membrane, reduced surgical time, lesser cost and the ease and potential to treat periodontal intrabony defects spanning more than 2 teeth.

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