A PROTOCOL TO STUDY TISSUE REGENERATION IN ALVEOLAR BONY DEFECTS

by

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DECLARATION

I hereby declare that this dissertation is my own work. It is being submitted for the degree of Master of Dentistry in the branch of Periodontology and Oral Medicine at the University of Pretoria. It has not been submitted before for any degree or examination at this or any other University.

André Christiaan Hattingh

This dissertation is dedicated to my wife, Werda.

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ABSTRACT

The ultimate goal of periodontal therapy remains to provide a dentition that remains in health and comfort for the life of the patient. This goal is now within reach, even in patients with destructive periodontal diseases, since the periodontal apparatus can be regenerated in infrabony defects implanted by various biocompatible materials. The ideal bone replacement implant should be able to trigger osteogenesis, cementogenesis and formation of a functional periodontal ligament. Most bone replacement implants are osteoconductive, relatively inert filling materials, and integrate with new bone. Histologically, these bone replacement implants have produced limited regeneration. Six types of alloplastic materials are now commercially available and their use has been regularly reported in the literature for the past four decades. It is, however, evident that synthetic implant materials function primarily as biocompatible defect fillers and, if regeneration is the desired treatment outcome, other materials should be used. Autogenous bone grafts provide both a potential for bone fill and regeneration, but are less frequently used due to the morbidity associated with a second surgical procedure to obtain the graft material, and subsequent to observations that these grafts may induce ankylosis and root resorption. Allo-implants have been used extensively in periodontal reconstructive surgery and, although controversy surrounded the osteoinductive potential of these implants, there is convincing histological evidence of regeneration with demineralized freeze-dried bone allo-implants. Guided tissue regeneration has made regeneration of the periodontium more predictable, but the ideal augmentation material remains to be found.

Bone morphogenetic proteins (BMPs) have the correct properties to induce regeneration of the periodontium. Animal and human studies have shown the potential of BMPs to act as the ideal periodontal regenerative material. It is possible that synergy between various BMPs potentiate the effect that they have individually on the extent of regeneration. Indeed a synergistic interaction has been documented in non-human primates between members of the transforming growth factor-β superfamily and should now be studied in humans.

This dissertation includes a protocol for a multi-centre study of twenty patients per centre. Native bone derived bovine BMPs, added to a human insoluble collagen bone matrix as carrier will be used to evaluate its regenerative potential in periodontal infrabony defects in humans. Such a study may see periodontal regeneration in a predictable state, and may advance our knowledge of surgical applicability of bone inductive materials.

OPSOMMING

Die mees gesogte doel in periodontale behandeling bly steeds die behoud van 'n natuurlike gebit in gesondheid en gemak vir die totale lewensduur van die pasiënt. Hierdie doel is nou bereikbaar, selfs in pasiënte met vernietigende periodontale siekte, omdat die periodontale infra-benige defekte geregenereer kan word deur verskeie bio-aanvaarbare materiale. Die ideale beenvervangingsinplantaat moet in staat wees om osteogenese, sementogenese en formasie van 'n funksionele periodontale ligament te inisiëer. Die meeste beenvervangingsinplantate is osteo-konduktiewe, relatiewe inerte vuller-materiale en integreer met nuwe been. Histologies het hierdie beenvervangingsinplantate min bewyse van ware regenerasie opgelewer. Ses tipes alloplastiese materiale is nou kommersiëel beskikbaar en hul aanwending was dikwels gerapporteer in die literatuur van die afgelope vier dekades. Dit is egter duidelik dat sintetiese inplantaatmateriale hoofsaaklik as bio-aanvaarbare defekvullers funksioneer en indien regenerasie die verlangde behandelingsdoel is, behoort ander materiale gebruik te word. Outogene beentransplantate bied beide 'n potensiaal vir beenvul en regenerasie, maar word minder algemeen gebruik weens die hoë morbiditeit geassosieer met 'n tweede chirurgiese prosedure om die transplantaatmateriaal te verkry. Hierdie materiale word ook geassosieer met ankilose en wortelresorpsie. Allo-inplantate word dikwels in periodontale rekonstruktiewe chirurgie gebruik en alhoewel kontroversie bestaan rondom die osteo-induktiewe potensiaal van hierdie inplantate, is daar oortuigende histologiese bewyse van regenerasie met gedemineraliseerde gevriesdroogde been allo-inplantate. Gerigte weefselregenerasie het regenerasie van die periodontium meer voorspelbaar gemaak, maar die ideale augmentasie materiaal moet nog gevind word.

Been morfogenetiese proteïene (BMPs) beskik oor die korrekte eienskappe om regenerasie van die periodontium te induseer. Diere- en mense studies het die potensiaal van BMPs om as die ideale periodontale regeneratiewe materiaal op te tree, reeds bewys. Dit is moontlik dat sinergisme tussen verskeie BMPs die effek wat hulle individueel mag hê, kan potensiëer. 'n Sinergistiese interaksie is reeds in nie-menslike primate tussen lede van die transformerende groeifaktor-β superfamilie aangetoon en behoort nou in mense bestudeer te word.

Hierdie verhandeling sluit 'n protokol in vir 'n multi-sentrum studie van ongeveer twintig pasiënte per sentrum. Oorspronklike been-afkomstige bees BMPs, gevoeg by menslike onoplosbare kollageen beenmatriks as draer, sal gebruik word om die regeneratiewe potensiaal daarvan in infrabenige menslike periodontale defekte te evalueer. So 'n studie mag toon dat periodontale regenerasie 'n voorspelbare prosedure is en mag ons kennis van die chirurgiese aanwending van been-induktiewe materiale verryk.

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LIST OF ABBREVIATIONS

AA	-	amino acid
BBD	-	BMP Bone Device
BCP	-	biphasic calcium phosphate
BMPs	-	bone morphogenetic proteins
BRD	-	bone regenerative devices
CEJ	-	cemento-enamel junction
DBM	-	demineralized bone matrix
DFDBA	-	decalcified (demineralized) freeze-dried bone allo-implants
DFDBI	-	demineralized freeze-dried bone allo-implant
ePTFE	-	expanded polytetrafluoroethylene
FDBA	-	freeze-dried bone allo-implants
GTR	-	guided tissue regeneration
HA	-	hydroxyapatite
HR	-	high resolution
HTR	-	hard tissue replacement
ICBM	-	insoluble collagen bone matrix
OP-1and OP-2	-	osteogenic protein-1 and 2
PD	-	probing depth
PDL	-	periodontal ligament
PTFE	-	polytetrafluoroethylene
rhBMP	-	recombinant human BMP
TCP	-	tricalcium phosphate
TGF-β	-	transforming growth factor-β

CHAPTER 1: REVIEW OF THE LITERATURE

1.1. BACKGROUND INFORMATION

1.1.1. Definition

Periodontitis is defined as an inflammation of the supporting tissues of the teeth. It is usually a progressively destructive change that leads to loss of bone and periodontal ligament. It is characterized by an extension of inflammation from gingiva into adjacent bone and ligament.[27]

1.1.2. History

Before the 1950s, periodontitis was treated mostly by tooth exfoliation or extraction, and this is still the predominant method of treatment for most of the world's populations today. Debridement of the root surfaces by scaling and root planing came into relatively common use in the first half of the present century and has become the central feature held in common by all currently used forms of periodontal therapy. Until the 1980s, a commonly used treatment consisted of scaling and root planing, followed by resective surgery aimed at achieving near zero millimetre sulcular depth. During the 1980s, clinical data demonstrated that the major determinants of successful periodontal therapy were the thoroughness of root debridement and subgingival infection control, and were not the presence or absence of periodontal pockets. Subsequently, non-surgical therapy became a more commonly accepted method of treatment. Neither resective surgery nor non-surgical therapy result in significant regeneration of the periodontal attachment. With the

understanding that periodontitis is an infectious process, the use of antibiotics and other anti-infective agents came into common use as adjuncts to other standard therapies. The comprehension of the pathways, by which the soft and mineralized tissues of the periodontium are destroyed, may lead to novel therapeutic strategies. For instance, non-steroidal, anti-inflammatory drugs may be used to decrease alveolar bone destruction by blocking prostaglandin production, and chemically modified tetracyclines that chelate divalent cations, may be used to block tissue destruction by the metalloproteinases. Recent data clearly show that regeneration of the previously destroyed periodontal attachment tissues is biologically possible, and regeneration has become the goal of therapy for the 1990s. Osteoconductive and osteoinductive materials can, under favourable conditions, induce roughly the regeneration of 60 to 70% of the height or volume of bony lesions with concomitant improvement in the clinical conditions. Regeneration by grafting or implantation may be further enhanced by use of barrier membranes that exclude gingival fibroblasts and epithelium from the healing site. Still further enhancement seems to be possible by local application of various growth factors, although studies in this important area are now only in their infancy. The future of periodontal therapy is exceedingly bright. It seems likely that we may be able to achieve nearly complete regeneration of the periodontal attachment apparatus at many sites, although not all, through combined therapy. To resolve the inflammatory process, to arrest the progression of the disease, and to induce the regeneration of the periodontal attachment tissues, the therapy would consist of root debridement, administration of anti-infective and anti-inflammatory drugs, and agents that inhibit metalloproteinases, followed by the combined use of implant materials, such as barrier membranes, and growth factors.[106]

1.1.3. Morphology of Alveolar Bony Defects

Although periodontitis is an infectious disease of the gingival tissue, changes that occur in bone are crucial because it is the destruction of bone that is responsible for tooth loss. Bone loss caused by extension of gingival inflammation is responsible for reduction in the height of the alveolar bone, whereas trauma from occlusion causes bone loss lateral to the root surface or may aggravate any type of periodontitis. Different types of bone defects can result from destructive periodontal disease. Cuneiform bony defects are those that occur in an oblique direction, leaving a hollowed out trough in the bone alongside the root. These defects are classified on the basis of the number of osseous walls, and may have one, two or three walls. Osseous craters are peripheral radicular concavities or defects in the crest of the interdental bone confined within the facial and lingual walls. The term furcation involvement refers to the extension of periodontitis into the furcation area of multirooted teeth.[2]

1.1.4. Therapy

All the above-mentioned bony defects have, in the past, been filled with various biocompatible materials in an attempt to regenerate lost bone. The materials can be classified as follows: (1) alloplastic implants, (2) bone autografts, (3) allo-implants, (4) xenogeneic implants, (5) absorbable and non-absorbable membranes for guided tissue regeneration (GTR) and (6) bone morphogenetic proteins (BMPs). In 1985 Lance[77] suggested that the term "graft" be reserved for tissue which is living and "implant" for all non-living materials, therefore this dissertation will follow the same terminology.

1.1.5. Terminology

The goal of periodontal therapy remains to provide a dentition that functions in health and comfort for the life of the patient.[26] Regeneration is defined as a reproduction or reconstitution of a lost or injured part.[27] It is, therefore, the biologic process by which the architecture and function of lost tissues are completely restored.[21] Periodontal regeneration is regeneration of the tooth's supporting tissues, including alveolar bone, periodontal ligament, and cementum. Repair means healing of a wound by tissue that does not fully restore the architecture or function of the part.[27] Periodontal regeneration is characterised by: 1) areas of complete regeneration (newly formed bone, cementum, and periodontal ligament), and 2) areas of repair including new connective tissue attachment (connective tissue adaptation or attachment via new cementum) and a new junctional epithelial attachment. Both repair and regeneration are mechanisms involved in the response to regenerative attempts around natural teeth.[21] New attachment means the reunion of connective tissue with a root surface that has been deprived of its periodontal ligament. This reunion occurs by the formation of new cementum with inserting collagen fibres. The formation of new bone is not necessarily a prerequisite for new attachment. New attachment to a root surface may be mediated through epithelial adhesion (junctional epithelium) or connective tissue adhesion. Reattachment means to attach again, i.e., the reunion of gingival connective tissue with a root surface on which viable periodontal tissue is present. The area of reattachment is not affected by bacterial contamination. Bone fill is the presence of hard tissue in a periodontal osseous defect, as determined by clinical examination in the course of a re-entry of the original defect site. This term does not indicate the nature of the histologic attachment to the tooth. The amount of bone fill is usually determined by surgical re-entry procedures.[27,78]

1.1.6. Bone Replacement Implants

The ideal bone replacement implant should be able to trigger osteogenesis, cementogenesis and formation of a functional periodontal ligament. Osteogenesis, the formation of mineralized bone by transplanted osteoblasts, is only achieved with autogenous grafts.[36] Cellular elements or progenitor cells of the autogenous graft have to be present for this to occur. Other types of bone replacement implants do not provide any cellular elements. The best scenario for these bone replacement implants would be osteoinduction, which is the stimulation of phenotypic conversion of progenitor cells within the healing wound to those that can form osseous tissue. Currently, demineralized freeze-dried human bone and bone morphogenetic proteins may fulfil this role.[1, 107]

Most bone replacement implants are osteoconductive, relatively inert filling materials, and integrate with new bone. Osteoconductive materials provide a scaffold to allow bone ingrowth and deposition and may support significant improvement in clinical probing depth and attachment levels. Histologically, these bone replacement implants have produced limited regeneration.[95]

Several alloplastic implant materials have been used to regenerate bone in periodontal infrabony defects. A review of these materials and of other methods to regenerate alveolar bone will precede the review on BMPs.

1.2. ALLOPLASTIC IMPLANT MATERIALS

The available alloplastic implant materials are usually classified as resorbable or non-resorbable (Table 1). In general terms, plaster of Paris, calcium carbonate, tricalcium phosphate and resorbable hydroxyapatite undergo partial or total resorption in oral and periodontal surgery sites but the polymers and dense hydroxyapatites do not.[79]

Table 1 Alloplastic implant materials

PLASTER OF PARIS (historical interest)		Non-resorbable / Partially-resorbable
POLYMERS (HTR)*		Non-resorbable
BIOCERAMICS	1) **Resorbable**	
	Tricalcium phosphate	
	Resorbable hydroxyapatite	
	2) **Non-resorbable**	
	Dense hydroxyapatite	
	Porous hydroxyapatite	
	Bioglass	

*Hard Tissue Replacement

1.2.1. Plaster of Paris

In 1892, Dreesman[3] was the first to report on the use of plaster of Paris to fill bony defects in humans. In 1961, Peltier[4] utilised plaster of Paris to treat bony defects that were surgically created in experimental animals. This animal model constituted the basis that led to the treatment of various bony defects of orthopaedic origin in humans. He

concluded that the implantation of plaster of Paris in a bony defect is safe, and that healing regularly follows its absorption.

In 1971, Shaffer and App[5] applied the above-mentioned findings to treat human infrabony periodontal defects with plaster of Paris. In this well-conducted study, results proved that plaster of Paris does not induce any new bone formation in osseous defects. It is worth noting that, already 28 years ago, these authors stated that the key to successful elimination of bony defects by implants would only occur if cementum and periodontal ligament fibres were formed along with new bone.

It appears from the above-mentioned history that researchers, for over one century, have striven to find the ideal implant material that would regenerate periodontal bony defects. Although unusual implant materials such as Gelfoam[6], cartilage[7], dentin and cementum[8] have also been used with varying degrees of success or failure, six types of alloplastic materials are now commercially available and their use has been regularly reported in the literature for the past four decades. These include (1) porous tricalcium phosphate (TCP), (2) resorbable hydroxyapatite (HA), (3) dense HA, (4) porous HA, (5) HTR (hard tissue replacement) polymer (polymethylmethacrylate beads coated with polyhydroxy-ethylmethacrylate)[9] and (6) bioglass (Table 1).

Bioceramic allo-implants are composed primarily of calcium phosphate, with the proportion of calcium and phosphate similar to the one found in bone. The two most widely used forms are tricalcium phosphate and hydroxyapatite.[95]

1.2.2. Tricalcium Phosphate

Tricalcium phosphate, a porous type of calcium phosphate, is commonly used in the form of β-tricalcium phosphate. It serves as a biological filler, which is partially resorbable and allows bone replacement. Conversion of the implant is pivotal to periodontal regeneration; first, serving as a scaffold for bone formation, and then permitting replacement with bone.[108, 109]

Tricalcium phosphate as a bone substitute has gained clinical acceptance, but the results are not always predictable. In direct comparison with allogeneic cancellous implants (i.e., frozen allogeneic bone), the allogeneic implants appear to outperform tricalcium phosphate.[110] Although some bone deposition has been reported with tricalcium phosphate implants,[10, 11, 110, 111, 112] these particles generally become encapsulated by fibrous connective tissue and do not stimulate bone growth.[11, 111]

After implanting TCP in human periodontal bony defects, Baldock *et al.*[11] reported that TCP particles do not stimulate new bone growth. The TCP was walled off by fibrous connective tissue and it was unlikely that any new connective tissue attachment would be obtained.

In a study in which TCP was used to treat eight infrabony lesions in four patients, Stahl and Froum[10] removed *en bloc* the implanted areas 3 to 8 months after surgery (the time of harvesting has not been stated clearly by the authors). They reported no histological evidence of a new attachment, and that these implants were walled off by collagen and seemed to act as non-irritating fillers.

1.2.3. Hydroxyapatite

Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, is the primary mineral component of bone. Synthetic hydroxyapatites have been marketed in a variety of forms, primarily as a porous non-resorbable, a dense or solid non-resorbable, and a resorbable (non-ceramic) porous form. These above-listed properties are acquired during the processing of the basic calcium phosphate mixture. The temperature at which it is processed determines hydroxyapatite's resorbability. Resorbability is desirable if the intention is to have the implant eventually replaced by the host bone.[95]

1.2.4. Resorbable HA

Resorbable HA is a particulate material processed at a low temperature (OsteoGen, Impladent, Holliswood, NY; OsteoGraf LD, CeraMed Dental, LLC, Lakewood, CO). This HA is a non-sintered (non-ceramic) precipitate with 300 to 400 μm particles. It has been proposed that non-sintered hydroxyapatite resorbs and acts as a mineral reservoir for bone formation via osteoconduction as its slow resorption rate allows bone cells to use the particles as a scaffold for bone replacement.[113, 114]

1.2.5. Non-Resorbable HA

When prepared at high temperature (sintered), HA is non-resorbable, nonporous, dense, and has a larger crystal size.[115] Dense HA implants are osteophillic, osteoconductive and act primarily as inert biocompatible fillers.

In an experiment to assess the clinical and histological response to dense HA granules implanted in infraosseous lesions, Froum *et al.*[12] analysed the histology of specimens harvested between 2 and 8 months postimplant surgery. They reported only a decrease of pocket depth, but neither gain of periodontal attachment, nor osteogenesis and cementogenesis adjacent to the implanted particles. They concluded that the material was a biocompatible "fill". In a similar study, Stahl *et al.*[13] found that the bony defects implanted with HA showed limited periodontal regeneration identical to the regeneration occurring at sites treated with debridement and autogenous bone grafts. However, the latter treatment produced a faster regeneration. Again, they theorised that the HA implant acted as a "filler".

To test the efficacy of HA granules, Rabalais *et al.*[16] implanted the granules into periodontal infrabony lesions and used non-implanted defects as controls. At surgical re-entry 6 months later, alveolar bone level measurements indicated greater fill in the experimental sites. Consequently, they proposed that HA had a definite potential to treat periodontal osseous defects.

At nine months re-entry, in a similar experiment using HA granules to treat human periodontal osseous defects, Meffert *et al.*[17] suggested that HA is an alloplastic implant clinically accepted by soft and hard tissues.

HA granules yield similar defect fill as other bone replacement implants, and the clinical improvement is more stable than with debridement alone.[95] In this regard, Yukna *et al.*

demonstrated over a five-year period that surgical debridement was not stable and regressed three to five times faster than sites having been implanted with HA.[116]

1.2.6. Porous HA

Porous HA (Interpore 200, Irvine, CA) is obtained by the hydrothermal conversion of the calcium carbonate exoskeleton of the natural coral genus *Porites* into the calcium phosphate HA. It has a pore size of 190 to 200 μm, which allows bone ingrowth[117, 118] into the pores and ultimately within the lesion itself.[19]

It is noteworthy that in a HA-induced osteogenesis model developed to study the effect of geometry and pore size (250 and 500 μm) of HA on bone differentiation, two substrata, namely granular HA and blocks of HA in rod configuration were used. With the exception of an island of bone that formed in two implants of granular HA of 500 μm pore size, bone differentiation occurred in all blocks of HA in rod configuration of either pore size at both observation periods. The lack of bone formation in the granular HA reveals the critical role of geometry of the HA substratum in bone differentiation.[28]

A study by Stahl and Froum[25] showed histological evidence of bone within the porosities of hydroxyapatite (HA) implants in human periodontal defects. However, healing resulted in a long junctional epithelium with no regeneration of the periodontal apparatus. Furthermore, there was root resorption.

In a study to evaluate healing after 4 months, in surgically created periodontal defects in dogs, implanted non-resorbable porous blocks of HA still contained significant amounts of

proliferating fibrovascular tissue, while control sites were completely filled with new bone.[14] In essentially the same animal model, other investigators[15] obtained similar results with TCP, a porous particulate resorbable implant.

In depth investigations of porous HA, as an implant material in periodontal defects, have shown that the material provides more extensive healing than with other alloplastic materials used to date.[18,19] Porous HA implanted in periodontal osseous defects produced a significant reduction in probing depth and depth of osseous lesions, and gains in attachment levels when compared to control sites at a 6-month re-entry.[18] In a multi-centre study to compare the regenerative potential of porous HA to demineralized freeze-dried bone allo-implant (DFDBI), significant greater clinical improvement was noticed in interproximal vertical periodontal defects treated with porous HA than in those implanted with DFDBI.[20] From recent reviews on the use of synthetic materials, it has been concluded that they lead to significant reduction in probing depth and improvement of clinical attachment levels.[21] On a histological basis, however, they act almost exclusively as biologic fillers inducing little bone fill and very limited, if any, periodontal regeneration.[21] The benefit of porous HA may be limited[23] since the healing pattern associated with porous HA appears to be periodontal repair,[22] and since the long-term clinical effect of a non-resorbable implant material that fills a space is unknown.

1.2.6.1. Combinations of Alloplastic Implant Materials

Combinations of the two primary forms of calcium phosphate have been studied to benefit from the rapid resorption of β-tricalcium phosphate and the inert scaffold of dense HA. A

histological study reported that biphasic calcium phosphate supported active bone replacement from surrounding bone, which may have been triggered by macrophages.[108]

In a comparable experiment to treat periodontal infrabony lesions in humans with porous biphasic calcium phosphate (BCP) and dense HA, Ellinger *et al.*[29] reported bone growth in the BCP implanted sites, while sites implanted with dense HA showed no bone growth. A new periodontal attachment did not developed in either ceramic implanted sites.

In a recent study [24] on a newly developed calcium phosphate cement, it was found that there is no rationale to support the use of HA cement as an implant material in this particular formulation for the treatment of cuneiform infrabony periodontal defects.

1.2.7. Bioglass

There are two forms of bioactive glass currently available. PerioGlas® (Block Drug Co., Jersey City, NJ) and Biogran™ (Orthovita, Malvern, PA). Bioactive glasses are composed of CaO, NA_2O, SiO_2, P_2O_5 and bond to bone through the development of a surface layer of carbonated HA.[119, 120] When exposed to tissue fluids, bioactive glasses are covered by a double layer composed of silica gel and a calcium phosphate-rich (apatite) layer. The calcium phosphate-rich layer promotes adsorption and concentration of proteins utilized by osteoblasts to form a mineralized extracellular matrix.[121] It has been theorized that these bioactive properties guide and promote osteogenesis,[19, 104] allowing rapid formation of bone.[122]

In a study on bioactive glass to treat human infrabony periodontal defects, improved healing and clinical outcomes were demonstrated. However, the authors[30] admitted that synthetic implant materials function primarily as biocompatible defect fillers.

1.2.8. HTR (Polymers)

HTR™ Synthetic Bone (Bioplant, Norwalk, CT) is a biocompatible microporous composite of polymethylmethacrylate, polyhydroxylethylmethacrylate and calcium hydroxide.[95] However, improved clinical results with this bone replacement implant have not always been achieved.[123] Histologically, new bone growth has been found deposited on HTR™ particles.[124, 33, 126] Its hydrophilic properties enhance clotting, and its negative particle surface charge allows adherence to bone. It appears to serve as a scaffold for osteoconduction when in close contact with alveolar bone. Satisfactory clinical outcomes when implanted in bony defects justify its use as a biocompatible alloplastic bone substitute.[127, 128]

Various studies have reported on the improved clinical outcomes of HTR.[31, 32] In five patients, the histological examination of 11 infrabony periodontal lesions treated with HTR microbeads revealed, at 4 to 26 weeks after implantation, that the microbeads were surrounded by a connective tissue capsule. Limited bone formation was present at the periphery of some microbeads.[33]

1.2.9. Concluding Note

Company-generated and independent research reports on synthetic implant materials have consistently claimed clinical results comparable to those obtained with non-synthetic implant materials. Alloplastic implant materials may have their greatest usefulness as autograft extenders, being added to available autogenous bone to provide a sufficient total volume of implant material. They may also be used as carriers for growth factors, antibiotics or other substances. Synthetic bone implant materials offer promises in periodontal therapy, but they are far from a panacea. Clinically, they do not perform better than autogenous or allogeneic materials.[79]

It is evident that synthetic implant materials function primarily as biocompatible defect fillers. If regeneration is the desired treatment outcome, other materials are recommended.[21]

1.3. AUTOGENOUS BONE GRAFTS

Autogenous bone grafts used to treat human periodontal defects are harvested from either intraoral or extraoral sites.[34,35] There are several types of autogenous bone grafts that have been or are being used clinically. They include cortical bone chips, osseous coagulum, bone blend, intraoral and extraoral cancellous bone, and marrow.[78] Extraoral autogenous bone grafts (iliac cancellous bone and marrow) may represent the greatest potential for bone growth.[34,35]

Table 2 Autogenous Bone Grafts

Intraoral	Extraoral
cortical bone chips	
osseous coagulum	
bone blend	
cancellous bone and marrow	

The use of autogenous bone grafts in periodontal therapy can be traced to the work of Hegedus (1923).[80] He reported success, in six cases of "advanced pyorrhea", by transplanting autogenous bone from the tibia to the jaws.

1.3.1. Extraoral Bone Grafts

There have been numerous reports of iliac crest cancellous bone and its associated marrow elements placed into periodontal osseous defects.[36,38,39,40,41,42.]

An extensive series[36] of case reports with hip marrow grafts revealed bone fill of 3.3 to 3.6 mm in 182 osseous periodontal defects, as well as 2.5 mm gain in crestal bone height (zero-wall defects).

Histological evaluation[37] of sites treated with iliac cancellous bone grafts consistently showed regeneration of bone and cementum, and functionally oriented periodontal

ligament fibres. New cementum deposition, osteogenesis around the grafted osseous spicules and along the walls of the osseous defects, as well as the rapid replacement of the implanted marrow by the host connective tissue, would suggest that the grafts enhance both osteogenesis and cementogenesis.

In a human study, Dragoo and Sullivan[38] presented histological evidence of crestal bone apposition, periodontal ligament formation and cementogenesis that strongly indicates periodontal regeneration to some coronal extent.

Today, iliac cancellous bone grafts have limited clinical use, although clinical and histological reports imply both a potential for bone fill and regeneration.[21] These grafts are now less frequently used due to the morbidity associated with a second surgical procedure to obtain graft material, and subsequent to the observation that the graft may induce ankylosis and root resorption, although the reported frequency is 5% or less.[37,43] Hiat *et al.*[37] reported that in over 6 300 sites treated with illiac cancellous bone grafts over a 16-year period, only one case had radiographic or clinical indication of ankylosis. Root resorption as observed by probing or radiographic examination, was noted before the first year postsurgery, except in two cases in which resorption was detected 6 years postsurgery. Schallhorn *et al.*[36] noted that root resorption, at 5 – 6 months after transplantation occurred in two out of 182 transplants and, in both instances, the cores were implanted within 3 hours following removal from the iliac crest. Schallhorn[43] speculated that hematopoietic marrow could possibly be treated to maintain its bone induction properties and to inhibit its dentinoclastic activity. In this regard, he suggested that the freshly harvested autogenous bone should first be frozen and then stored for use

at a later stage. Dragoo and Sullivan[38] reviewed over 250 fresh iliac cancellous bone autografts on humans and reported only a 2.8% prevalence of root resorption. These specimens were removed for histologic evaluation at 2-, 3-, 4-, 6-, and 8 month intervals.

1.3.2. Intraoral Bone Grafts

Intraoral bone graft material can be harvested from the maxillary tuberosity, the mandibular retro-molar area, healing extraction sites, the recontouring of bone at surgical sites (osseous coagulum), as well as from various other areas.

1.3.2.1. Cortical Bone

The impetus for the modern-day use of periodontal bone grafts can be traced to the work of Nabers and O'Leary (1965).[81] They reported that shavings of cortical bone removed by hand chisels during osteoplasty and ostectomy could be used successfully to attain a coronal increase in bone height. The authors felt that the one- and two- walled infraosseous defects they treated were not amenable to other methods of treatment. Cortical chips, due to their relatively large particle size (1559.6 x 183 μm)[82] and potential for sequestration, were subsequently replaced by autogenous osseous coagulum and bone blend.[78]

1.3.2.2. Cancellous Bone

Hiatt and Schallhorn treated 166 sites with intra-oral cancellous bone and marrow grafts. They reported an average bone fill of more than 50% of the volume of the treated defects and a 3.4 mm gain of alveolar bone height.[44]

In a similar study, Ellegaard and Löe treated 191 infrabony defects with intra-oral cancellous bone and marrow grafts. They reported that, within six months, 80% of three-wall defects and 70% of two-wall defects healed either completely or to over half of the original depth of the defects.[45] In contrast, a study by Renvert *et al.*,[46] on intra-oral osseous grafting of angular bony defects, resulted in a disappointing average of 1 mm gain of probing attachment and probing bone levels.

1.3.2.3. Osseous Coagulum and Bone Blend

Intra-oral bone, when obtained with high- or low-speed round burs and mixed with blood, becomes a coagulum.[83,84] The bone blend technique was designed to overcome some of the disadvantages of osseous coagulum, including inability to aspirate during the collection process, unknown quantity and quality of collected bone fragments, and fluidity of the material.[85] Bone blend is cortical or cancellous bone that is harvested with a trephine or rongeurs, placed in an amalgam capsule, and triturated to the consistency of a slushy osseous mass. The resultant particle size is in the range of 210 x 105 μm.[82]

A clinical investigation was undertaken to compare the regeneration of osseous defects with open debridement alone and with osseous coagulum-bone blend grafts. The average bone fill was only 0.66 mm for the open debridement procedure, but 2.98 mm for the grafting procedure.[47]

In the treatment of human periodontal osseous defects with hip marrow autografts and intraoral osseous coagulum-bone blends, 60.7% and 73% of bone fill were respectively

obtained.[48] The histological examination of the grafted sites revealed regeneration of the periodontal apparatus and new connective tissue attachment coronal to notches made in calculus.[13,50]

The healing sequence of an autogenous periodontal bone graft has been identified as initiation of new bone formation at 7 days, cementogenesis at 21 days, and a new periodontal ligament at 3 months.[78] By 8 months, the graft should be incorporated into host bone with functionally oriented fibers coursing between bone and cementum. Maturation may take as long as 2 years.[78, 38]

1.4. ALLO-IMPLANTS

Three types of bone allo-implants are available from tissue banks.[21] These include frozen iliac marrow and cancellous bone, mineralised freeze-dried bone allo-implants (FDBA), and decalcified (demineralized) freeze-dried bone allo-implants (DFDBA).

Table 3 Types of bone allo-implants

Frozen iliac marrow and cancellous bone
Mineralised freeze-dried bone allo-implants (FDBA)
Decalcified (demineralized) freeze-dried bone allo-implants (DFDBA)

1.4.1. Frozen Iliac Marrow and Cancellous Bone

The need for extensive HLA typing to decrease the likelihood of allo-implant immune reaction, as well as the risk of infectious disease transmission, has virtually eliminated the use of frozen iliac allo-implants in periodontics.[21,51]

In a recent study to treat infrabony alveolar defects with cryo-preserved allo-implants from femur head, bone fill of 1.8 mm (60% defect fill) was achieved for implanted sites while control sites showed a bone fill of 0.6 mm (29%) only.[54]

1.4.2. Mineralized Freeze-Dried Bone Allo-Implant

Mineralized FDBA was introduced to periodontal therapy in 1976.[88] Freeze drying removes approximately 95% of the water from bone by a process of sublimation in a vacuum. Although freeze drying kills all cells, the morphology, solubility, and chemical integrity of the original specimen are relatively well maintained.[23, 89, 90,] Freeze drying also markedly reduces the antigenicity of a periodontal bone allo-implant.[91,92] At no time could any donor-specific anti-HLA antibodies be detected in any human recipient who received several FDBA.[92]

In 1991, Mellonig reviewed the data resulting from the use of FDBA in periodontal reconstructive surgery.[23] In all, 89 clinicians treated 997 sites with FDBA alone and 524 sites with FDBA plus autogenous bone. For clinical evaluation, 327 sites treated with FDBA only and 176 sites treated with FDBA plus autogenous bone were re-entered surgically. Complete or greater than 50% bone fill was achieved in 220 (67%) sites treated with FDBA only, and in 137 (78%) sites treated with FDBA plus autogenous

bone. He concluded that FDBA plus autogenous bone was more effective than FDBA alone, particularly in furcation defects.

Altiere *et al.*[53] treated 10 paired defects either with FDBA or debridement. In both experimental and control sites, the average of bone fill was greater than 50% in 60% of the defects. No significant differences could be found between both groups.

1.4.3. Demineralized Freeze-Dried Bone Allo-Implant

Animal studies carried out by Urist and co-workers[55, 56, 57, 58] and replicated by others[59, 60, 61] have shown that demineralization of a cortical bone allo-implant (DFDBA) enhances its osteogenic potential by exposing bone inductive proteins collectively called bone morphogenetic proteins (BMPs).[62] These osteoinductive proteins induce undifferentiated host cells into osteoblasts.[63] In treatments with a non-demineralized allo-implant, osteoinduction is absent, but instead osteoconduction may occur along the implant that acts as a scaffold for the formation of new bone.[64]

The sequence of bone induction with a DFDBA is believed to follow a bone induction cascade.[93,94] At day 1, there is chemotaxis of mesenchymal cells with attachment to the DFDBA. At day 5, there is continued cell proliferation and differentiation into chondroblasts. At day 7, chondrocytes synthesize and secrete matrix. From days 10 to 12, there is vascular invasion, differentiation of osteoblasts and bone formation, and mineralization. By day 21, there is bone marrow differentiation. This cascade for the induction of endochondral bone has been shown to occur in heterotopic sites of animals implanted with demineralized bone matrix.[93]

In severe periodontal defects, Libin *et al.* implanted decalcified lyophilised bone allo-implants prepared as described by Urist.[65] They reported that both cortical and cancellous types of the above-mentioned implants resulted in new bone formation and in a gain of attachment level.

Controlled clinical trials have shown greater bone fill in sites treated with DFDBA than in non-implanted sites.[66]

DFDBA has been compared to FDBA in the treatment of 11 paired infra-osseous defects. A re-entry procedure 6 months post-surgery revealed no statistical differences in any of the parameters including attachment level gain, bone fill and probing depth reduction.[67]

In a study, Becker *et al.*[68] suggested that the quantity of active BMPs in a periodontal DFDBA implant was too small to induce bone formation, and that there was a need to supplement the collagenous matrix with recombinant human BMP (rhBMP). In an investigation, Shigeyamce *et al.* suggested that DFDBA does contain adequate quantities of active BMPs.[69] However, using identical volumes of fresh bone and DFDBA, they found that a larger quantity of BMPs could be extracted from fresh bone.

The osteoinductive potential of DFDBA is supported by a large database of previously published studies.[55, 56, 57, 58, 59, 60, 61, 70, 71, 72, 73, 74]

Histological evidence of regeneration with DFDBA implants is most extensive and conclusive in the periodontal literature and supports its positive impact on the regenerative process.[21]

Most human histological evaluations have been criticized because they failed to adequately demonstrate that the adjacent root surface was biologically contaminated and devoid of its connective tissue attachment. For example, histological evaluation is commonly done on the root surface from a notch made with a bur in the cementum at the base of the defect, to a notch placed in cementum at the level of the alveolar crest. All of these histological reference points, although highly suggestive of a root exposed to the oral bacterial contamination, do not prove that the root surface has lost its connective tissue attachment (periodontal ligament). Therefore, reattachment rather than regeneration might have been the result. Currently, only a notch placed in the most apical level of calculus on the root surface is considered scientifically valid proof of regeneration of an attachment apparatus.[78]

Periodontal regeneration above notches placed in calculus was consistently present when DFDBA was implanted both in open and submerged defects. Magnitude of bone fill was calculated from the base of the initial defect to the most coronal level of regenerated bone. There was a mean bone fill of 5.4 mm in the 32 reported non-submerged defects treated with DFDBA. This represents 79% of the mean volume of the original defects. Results indicated that in the submerged environment, regeneration was possible with and without the placement of a bone implant. However, more new attachment apparatus formed in implanted than non-implanted sites. In addition, new bone, new cementum, and

periodontal ligament occurred more frequently in implanted than non-implanted sites. These results strongly suggest that bone implants do have an inductive effect on the periodontium.[75, 76]

1.5. XENOGENEIC IMPLANTS

Table 4	Xenogeneic implants
	Bovine bone
	Natural coral

Xenoimplants are implants shared between different species. Currently, there are two available sources of xenoimplants used as bone replacement implants in periodontics; bovine bone and natural coral. Both sources, through different processing techniques, provide products, which are biocompatible and structurally similar to human bone. Xenoimplants are osteoconductive, readily available and risk free of disease transmission. The latter point has been questioned with the discovery of bovine spongiform encephalopathy, particularly in Great Britain.[95]

1.5.1. Bovine Bone

Commercially available bovine bone is processed to yield natural bone mineral minus the organic component. A purported advantage of these products is that they provide structural components similar to that of human bone, with improved osteoconductive

capability when compared with synthetic materials. Anorganic bovine bone is the hydroxyapatite "skeleton" that retains the macroporous and microporous structure of cortical and cancellous bone[96] remaining after chemical or low-heat extraction of the organic component. Historically, bovine xenografts have failed due to rejection,[97] probably because the organic components were extracted with chemical detergents, leaving residual protein and, therefore, produced adverse reactions and clinically unacceptable results.[98]

Currently available bovine-derived hydroxyapatite is deproteinated, retaining its natural microporous structure, which supports cell-mediated resorption.[96] This becomes important if the product is to be replaced with new bone. Bovine-derived hydroxyapatite bone replacement implants 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 them to integrate with host bone. They have been used with success for the treatment of intrabony defects and ridge augmentation.[99]

1.5.2. Natural Coral

Biocoral (Inoteb, Saint Gonnery, France) is calcium carbonate obtained from a natural coral, genus *Porites,* and is composed primarily of aragonite (>98% $CaCO_3$). Its pore size of 100 to 200 μm is similar to the porosity of spongy bone.[100] Its porosity provides a large surface area for resorption and replacement by bone.[101] Unlike porous hydroxyapatite, derived from the same coral by heat conversion and made non-resorbable, calcium carbonate is resorbable. It does not require a surface transformation into a carbonate phase as do other bone replacement implants to initiate bone formation;

hence,[100] it should more rapidly initiate bone formation. Biocoral has a high osteoconductive potential because no fibrous encapsulation has been reported.[102] Coralline calcium carbonate produces comparable results to other bone replacement implants with significant gain in clinical attachment, reduction of probing depth and defect fill.[103, 104, 105]

1.6. GUIDED TISSUE REGENERATION

Table 5	Guided Tissue Regeneration
	Non-absorbable barriers
	Absorbable barriers

1.6.1. Clinical Assessment

With the advent of guided tissue regeneration (GTR), restoration of the periodontium is being achieved more predictably. GTR is based upon the biological behaviour of different periodontal tissues during wound healing. Placement of a subgingival barrier achieves the following: (a) epithelial cells are impeded from apically migrating and interfering with connective tissue-root surface interactions; (b) gingival connective tissue from the flap is excluded from healing sites; and (c) progenitor cells from the periodontal ligament and the marrow spaces of the alveolar bone are favoured to repopulate the coronal root surface, thereby facilitating formation of a new periodontium.[129]

The technique using barriers was introduced by Nyman *et al.*[130] in 1982 and the term GTR was coined by Gottlow *et al.* in 1986.[131] This method of enhancing periodontal regeneration was also referred to as selective cell repopulation[132] or controlled tissue regeneration.[133] Histological evaluations in animal models confirm that significant amounts of new periodontium is regenerated by the technique of GTR.[130, 132, 133, 134, 135, 136, 137] Case reports and human clinical trials have supported the contention that GTR reduces probing depths and favours a gain of clinical attachment.[138, 139, 140] However, it is unresolved as to whether improved periodontal status is due to barrier-guided cells or protection of the initial blood clot.[141, 142]

Histological and clinical assessments reveal that incomplete regeneration of defects frequently occurr with GTR. Aukhil *et al.*[134] noted that 3 zones of healing developed after barrier placement: junctional epithelium, fibers parallel to the root surface and new connective tissue attachment (bone, cementum and periodontal ligament). Others reported new soft tissue attachment without concomitant bone deposition.[138, 139, 143] Wide variability of results between studies may be attributed to the architecture of bone defects being treated, duration of healing, type of barrier used, flap position, recession, plaque control, the systemic health of the patient and other factors.[135, 144]

1.6.2. Historical Background

The first clinical device used in periodontal surgery, which allowed regeneration of cementum, periodontal ligament and alveolar bone was a cellulose acetate (paper) laboratory filter.[130,147] The use of the paper filter provided the first human histological evidence of periodontal regeneration.[147] This barrier, however, lacked several

characteristics deemed necessary for guided tissue regeneration. Since then, several barriers made out of a variety of materials have been introduced.[148]

Initially, non-resorbable barriers (Millipore filters and polytetrafluoroethylene) were used to facilitate GTR.[145] However, the use of these materials dictated secondary surgical procedures for removal, and this reduced the practicality of using GTR during routine patient management. To avoid this dilemma, investigators assessed the efficacy of biodegradable barriers.[129] A major distinction can be made between two types of devices; non-absorbable and absorbable (biodegradable) devices.[148]

1.6.3. Non-Absorbable Barriers

Non-absorbable barriers were the first devices approved for clinical use. They maintain their structural integrity and, consequently, the essential features they possess, for as long as they are left in the tissues. This compositional and design stability provides the operator with complete control over time of application, with the potential to minimise variation in effectiveness. Non-absorbable barriers require, by their very nature, a second surgical procedure for removal.[148]

Most non-absorbable devices are made from polytetrafluoroethylene (PTFE)or expanded polytetrafluoroethylene (ePTFE). PTFE is a fluorocarbon polymer with exceptional inertness and biocompatibility.[148] ePTFE is PTFE subjected to tensile stress during manufacture, resulting in differences in physical structure. The potential of these ePTFE devices to support periodontal regeneration has unequivocally been demonstrated in canine and non-human primate studies.[133, 150, 151]

Observations of histological specimens from human cases indicate that ePTFE barriers may support significant amounts of periodontal regeneration following a three-month healing interval.[131] Another human case demonstrated new cementum with inserting fibers at six months following reconstructive surgery including an ePTFE membrane.[153] The efficacy of ePTFE barriers in different clinical settings has been evaluated.[154,155] The use of ePTFE devices has been associated with minor complications such as pain, purulence, swelling, and tissue sloughing, with an incidence slightly higher than that reported for conventional periodontal surgery.[156]

1.6.4. Absorbable Barriers

Absorbable barriers do not require additional surgery for removal, which reduces patient discomfort, chair-side time and related cost, while eliminating potential surgery-related morbidity.[148] Reviewing the biological rationale for guided tissue regeneration, Minabe[145] concluded that absorbable devices should maintain their *in vivo* structure for at least four weeks. Robert *et al.*[158] suggested that longer time periods may be necessary. Because of their biodegradability, absorbable devices elicit inevitable and necessary tissue reactions that may influence wound healing. Ideally, these inflammatory reactions should not compromise the intended regenerative outcome.[148]

Absorbable materials used for guided tissue regeneration devices fall into two broad categories: natural products (collagen) and synthetic materials. These two groups will be addressed separately.

1.6.5. Collagen Barriers

Tissues such as tendon, bone and skin, have a uniquely high type I collagen content. Collagen accounts for almost 60% of gingival connective tissue[159] and 90% of total protein in bone.[160] Intrinsic collagen participates in soft tissue and bone healing.[161] Exogenous collagen exhibits hemostatic activity,[162] is able to attract and activate neutrophils[163] and fibroblasts,[164] and interacts with various cells during tissue remodelling and wound healing.[165] These biological activities, along with low immunogenicity,[166] make collagen an attractive biomaterial.[167]

Collagen used for medical devices is derived from several animal sources including bovine skin, tendon, intestine, or sheep intestine.[148] Implanted collagen devices are primarily degraded by the enzymatic activity of infiltrating macrophages and polymorphonuclear leukocytes.[168] The potential of collagen membranes for guided tissue regeneration has been evaluated in animal studies. A bovine collagen membrane resorbed within eight weeks,[169] and a rat-tail collagen membrane resorbed within four weeks of surgery.[170]

A type I collagen guided tissue regeneration membrane approved for clinical use is manufactured from collagen derived from bovine deep flexor (Achilles) tendon (BioMend™). This membrane is semi-occlusive and completely absorbed in four to eight weeks. As evaluated in clinical settings, the performance of the membrane appears to vary depending on the type of defect being treated.[171,172] Collectively, collagen-based devices appear to have limited, if any, potential to support guided tissue regeneration, as evaluated in various animal models and clinically, primarily because of their limitations in providing/maintaining a wound space.[148]

1.6.6. Synthetic Absorbable Barriers

1.6.6.1. Chemical Composition

Synthetic absorbable devices for medical use are usually manufactured from organic aliphatic thermoplastic polymers. The materials most commonly used are poly(α -hydroxy acids), which include poly(lactic acid), poly(glycolic acid), and their copolymer(s) poly(glycolide-lactide). Poly(glycolic acid) and poly(lactic acid) are manufactured by catalytic polymerization of the monomers and are widely used for sutures and drug controlled-release devices.[148] Several aspects of the material properties of poly(α-hydroxy acids) have been reviewed.[173,174,175] One apparent advantage of poly(α-hydroxy acids) is their degradation by hydrolysis, resulting in decomposition products that are mostly metabolised to carbon dioxide and water through the citric acid (Krebs) cycle.[176]

1.6.6.2. Other Available Barriers

A double-layered absorbable device, GUIDOR® matrix barrier, made of poly(lactic acid) (containing both L- and D-lactic acid enantiomers) and a citric acid ester (acetyl-tributylcitrate) was the first to gain FDA approval.[177] Histological studies in animals suggest that this device is completely absorbed by 6 to 12 months post-implantation and maintains its barrier function (structure) for at least 6 weeks post-implantation.[178] The degradation process in non-human primates involves the typical foreign-body reaction characterized by macrophages and multinucleated giant cells, observed 3 and 6 months post-implantation.[179]

Another synthetic absorbable device, RESOLUT® Regenerative Material, is a composite consisting of an occlusive membrane of glycolide and lactide copolymer and a porous web structure of bonded polyglycolide fiber.[148] Histological studies indicate that this device is as effective as non-resorbable devices, retains its structure for four weeks and absorbs completely within five to six months post-implantation.[181]

Fiber of polyglactin 910, a copolymer of glycolide and L-lactide (90/10 molar ratio), is used to prepare a tightly woven mesh (VICRYL® Periodontal Mesh). Evidence suggests that this device can lose integrity within two weeks and resorb within four or more weeks, depending on the host species.[182] Although recession and lack of tissue integration have been reported in animal studies,[183, 178] clinical evaluation of the polyglactin 910 device suggests that it is not less effective than other devices in a variety of defects.[182, 209]

The ATRISORB® barrier is the only approved guided tissue regeneration device to be manufactured chair-side.[148] The poly(DL-lactide) polymer is supplied in a flowable formulation, dissolved in *N*-methyl-2-pyrrolidone.[148] The polymer accounts for 37% of the formulation and the solvent for 63%, by weight. An irregularly shaped barrier is formed after exposure of the polymer to 0.9% saline solution for four to six minutes in a special cassette. Histological observations suggest that the device is completely absorbed by six to twelve months.[184] The clinical efficacy of this device has been evaluated with favourable results.[185, 186]

In addition to the more commonly used polyesters described above, studies have evaluated the potential of other absorbable barriers based on polyurethane.[187] Polyurethanes are organic polymers containing the urethane group, -NH-CO-O-, encompassing a variety of materials with diverse properties, such as high tensile strength, lubricity, good abrasion resistance, ease of handling, and good biocompatibility.[188]

1.6.7. Concluding Discussion on Barriers

When evaluating clinical results of GTR studies around natural teeth, evidence seems conclusive that there is a clear difference in favour of GTR when compared to debridement when treating mandibular Class II furcation defects. Maxillary Class II defects have responded less favourably with no advantage to GTR procedures compared to debridement in interproximal sites and improvement beyond debridement in buccal sites. Mandibular and maxillary Class III defects treated with GTR have shown only minimal improvements. When GTR procedures are used in infraosseous defects, clinical results are better than with debridement alone. The database however is not as strong as that associated with Class II furcations. Additional research is necessary.[21]

There are no data to suggest a clinical advantage associated with the use of one barrier membrane versus another. Comparative studies between barrier devices have demonstrated similar clinical outcomes following a multitude of different comparisons.[21]

The ideal augmentation material remains to be found. Such a material should induce osteogenesis and cementogenesis that should result in regeneration of a new periodontal attachment complex at a more coronal level. It should be completely biocompatible and

should not be carcinogenic, toxic, antigenic, or effect inflammatory responses. It should also be easily obtainable, relatively inexpensive and should not cause the patient or the therapist unnecessary inconvenience.[79]

1.7. BONE MORPHOGENETIC PROTEINS

1.7.1. Historical Background

In Urist's classic article at 1965 entitled "Bone: formation by auto-induction," the principle of induction is used to explain the results obtained upon implanting decalcified bone matrix.[49] Induction is defined as "an interaction between one (inducing) tissue and another (responding) tissue, as a result of which the responding tissue undergoes a change in its direction of differentiation".[180] Factors influencing the inductive process include the timing of the response (considering both the exposure time required as well as the time the inducer is capable of inducing), the localisation or proximity of competent cells able to respond, and the concentration.[49] Variations in each of these factors determine what, if any, effect is seen. In terms of osteoinduction, then, there must be inducing substances (bone morphogenetic proteins) acting upon a responding cell (an undifferentiated mesenchymal cell) to become an osteoprogenitor cell capable of forming bone.[49]

For over 30 years now, research has been carried out to isolate and purify BMPs, proteins which have been shown to induce heterotopic bone formation in various animal species. Recent advances in the fields of developmental biology, molecular biology, genetics and wound healing, have shown that the BMPs are not only responsible for postfetal bone

induction (including normal bone remodelling, healing and repair), but are also critical during embryogenesis, not only in regards to the skeletal system, but quite possibly in the morphogenesis and pattern formation of other tissues and organs as well. Therefore, BMPs have potential in orthopaedic and dento-alveolar reconstruction.[49]

Although bone grafts possess osteoconductive and osteoinductive properties, the latter albeit very limited, their usefulness and predictability is limited due to the difficulty in obtaining an adequate amount or specific size or shape of bone, as well as its frequent inability to functionally integrate into the bony defect. This, along with the current concern over the safety of transplanted human products, has led to the necessity of having available bone substitutes which incorporate the desired properties for bone growth and repair without the limitations and risks of materials presently available.[49]

1.7.2. Growth Factors

Many growth factors present in bone have been isolated and tested for their capability in causing bone growth.[52, 86] However, since growth factors act upon the osteoprogenitor (differentiated) cells present in developing or pre-existing bone, their effect is limited in large bony defects, and questionable in the generation of bone in the absence of osteoprogenitor cells. The advantage of utilizing differentiating morphogens versus growth factors in the (re)generation of bone is that the pre-existence of osteoprogenitor cells in the area may be unnecessary. The BMPs are the only morphogens "thus far found in mammalian species and are detected not only in the embryonic, but also postfetal bone development".[87]

1.7.3. Requirements for New Bone Formation

Of the many tissues in the body, bone has considerable potential for regeneration.[189] Bone formation requires three key components: the osteoinductive signal, a suitable substratum with which this signal is to be delivered and one which acts as a scaffold for new bone tissue to form, and responding host cells capable of differentiation into bone cells. Al three components are amenable to manipulation and form the essential ingredients for tissue engineering of bone as well as of the periodontal tissues.[189] The molecular basis of this regeneration is the family of the BMPs, isolated as a by-product of intense studies on the bone development cascade.[189] The principles gleaned from bone morphogenesis induced by BMPs can then be applied or adapted to the periodontal tissues.[189]

1.7.4. Brief Overview of Bone Induction Mechanism

The existence of BMPs was confirmed by Sampath and Reddi,[125] who observed that osteogenic activity could be restored by reconstituting the inactive insoluble collagenous bone matrix (ICBM) with solubilised protein fractions from demineralized bone matrix (DBM). This observation led to the purification, followed by expression cloning of the recombinant human BMPs.[190] Besides functioning as inductors of bone and cartilage formation, BMPs seem to play a critical role in development and regulatory function. For instance, specific BMPs are structurally similar to regulatory genes responsible for structural development in *Drosophila* organisms.[146]

It has been postulated that osteoblasts, cementoblasts, and their progenitors that are found in the periodontal ligament (PDL) may arise from the endosteal spaces of the alveolar process.[149, 152] Because *in vitro* studies suggest that BMPs can stimulate and maintain the

osteoblast phenotype, it may also be possible that similar effects will be seen on other cells isolated from the different components of the periodontal tissues.[202]

BMPs possess osteoinductive properties that stimulate the differentiation and proliferation of uncommitted mensenchymal stem cells into chondroprogenitor and osteoprogenitor cells. BMPs belong to the transforming growth factor-β (TGF-β) superfamily.[190] To date, 20 BMP sequences have been isolated. With the exception of BMP-1, which is not a member of the family, recombinant human (rh) BMP-2 through BMP-6 and osteogenic protein-1 and 2 (OP-1 and OP-2, also known as BMP-7 and BMP-8, respectively), in conjunction with a collagenous matrix as carrier, singly induce *de novo* bone formation when implanted into extraskeletal sites of a variety of animal models.[189] BMP-1, because of its amino acid (AA) sequences, cannot be classified as belonging to the TGF-β superfamily. It is not capable of inducing bone formation. However, it does have an AA sequence similarity with a crayfish protease, and also a sequence similarity to epidermal growth factor.[157] BMPs are the common molecular initiators deployed for embryonic development and induction of bone formation and regeneration in post-natal osteogenesis.[189]

Examination of the relationships between the BMPs has allowed the organization into three groups based on their AA sequence homologies. BMP-2 and BMP-4 are closely related proteins, having a strong homology in their AA domains. Similarly, BMP-5 through BMP-8 share a great deal of sequence homology and form another subgroup of the BMPs. These two subgroups (BMP-2/BMP-4 and BMP-5 through BMP-8) are more closely related to each other than they are to BMP-3 (osteogenin), which by itself forms

the third BMP subgroup. The BMPs, while residing in the TGF-β superfamily, constitute a distinct subfamily based on their AA sequences. In fact, they are related to the TGF-βs themselves (on the order of 30 to 35%), homology though they have quite distinct biological activities.[191]

It is well understood that there are many other substances which are responsible for bone growth and regulation.[49] However, presently it appears that growth factors are not capable of inducing bone formation in ectopic sites. Rather, they act to modulate or stimulate already determined osteoprogenitor cells to form cartilage and bone.[202] There is, therefore, a distinct difference between the ability and function of BMPs and growth factors.[49] There is evidence to support the synergistic activity of BMPs and growth factors. For example, we know that BMP implanted *in vivo* in ectopic sites will induce bone formation.[49] It would seem that the BMPs are responsible to initiate the differentiation of preprogenitor mesenchymal cells into chondrocytes, and bone derived growth factors and other processes promote and maintain the subsequent osteogenic cascade.[107, 202]

BMP-2, BMP-3 (osteogenin), BMP-4, BMP-5, BMP-6, osteogenic protein-1 (OP-1, BMP-7), and osteogenic protein-2 (BMP-8) have been implicated in cartilage and bone formation both *in vitro* and *in vivo*.[189, 190, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207]

1.7.5. Influence of Cellular Events

Following implantation of inductively active DBM or BMP, three phases of osteoinduction are observed, these being chemotaxis, mitogenesis, and differentiation.[93, 210] Chemotaxis initially brings polymorphonuclear leukocytes into the implant area, followed by fibroblasts and cell attachment to the matrix. In mitosis, there is proliferation of mesenchymal cells at day 3, as measured by [^{3}H]-thymidine incorporation into cellular DNA as well as an increase in ornithine decarboxylase activity in the implant.[93] The aggregation of mesenchymal cells coincides with an increase of Type I collagen mRNA, which may be an indicator of increased activity in these cells.[211] Differentiation of mesenchymal cells into chondroblasts takes place by day 5. This is believed to occur through the close matrix-cell interaction, and results in the synthesis of "extracellular matrix components typical of cartilage".[212] New cartilage can be quantitated by radiolablled $^{35}SO_4$ incorporation into cartilage-specific proteoglycan and immunofluorescent localization.[93] Following vascular invasion by day 9, maturation of chondrocytes and mineralization of cartilage takes place. Osteoblasts appear at days 10 through 12 and form new bone matrix while chondrocytes are active in removing the calcified cartilage. There is a peak in alkaline phosphatase production at this time by both chondrocytes and osteoblasts.[211, 212] From days 12 through 18, osteoclasts remodel the newly-formed bone and selectively dissolve the implanted matrix, resulting in an ossicle of new bone complete with marrow by day 21.[93] Apparently, the only biological difference between induced heterotopic bone and orthotopic bone is the lack of a true periosteum in heterotopic bone, which "might explain why such bones do not proliferate or regenerate after cessation of inductive stimuli".[213]

1.7.6. Bone Induction in the Periodontium

Periodontal regeneration entails *de novo* cementogenesis, osteogenesis, and generation of functionally oriented periodontal ligament fibers inserting into both newly formed cementum and alveolar bone.[214, 215] While regeneration of the periodontal tissues is the ultimate goal of the treatment of periodontitis, regeneration, as defined above, is not a predictable healing outcome in both experimental and clinical studies.[216, 217, 218] Rather, healing following surgical procedures devised for periodontal tissue regeneration proceeds predominantly *via* repair phenomena, in which the newly formed tissue does not fully restore the architecture of the lost tissue.[214, 215] This is because the regeneration of the periodontal tissues requires the functional union between connective and epithelial tissues with a completely avascular and almost impermeable root surface.[219, 220] In addition, healing phenomena occur in an environment in which several embryologically different and disparate cellular phenotypes contribute to the wound healing process, i.e., cells from the exposed root cementum, the periodontal ligament space, the residual alveolar bone, and the epithelial-connective tissue of the gingival unit[152, 214, 221] Each of these phenotypes must be capable of proliferation and migration to its specific "allocated" topographical zone, and must secrete extracellular matrix components which will define a specific type of periodontal tissue. From a cellular and molecular perspective, repair and regeneration of the periodontal tissues must be considered as a highly integrated process involving cell-to-cell and cell-extra-cellular matrix interactions. The synthesis and supra-molecular assembly of the extracellular matrix of the different periodontal tissues will ultimately determine the extent of regeneration of the affected periodontal unit.[189]

Several experiments have shown that the crux of periodontal regeneration lies in the early induction of cementogenesis and the assembly of newly formed periodontal ligament fibers onto the highly mineralized and avascular root surface. [147, 214, 217, 219, 220]

1.7.7. Experimental Procedures

In a pilot study with four adult baboons, Ripamonti *et al.*[205] reported that naturally-derived BMPs in conjunction with collagenous matrix induced cementum, periodontal ligament, and alveolar bone regeneration in surgically created defects. Bovine BMP fractions purified more than 50,000-fold with respect to initial crude extract, with an apparent molecular mass range of 26-42 kDa on SDS-PAGE and with osteogenic activity in the subcutaneous space of the rat were used for experiments in the primate.[205] Previous studies have shown that there is homology of osteogenic proteins among mammals[222] and that bovine BMPs, in conjunction with baboon collagenous matrix, induce bone differentiation in extraskeletal sites of the baboon with biological activity comparable with that of baboon BMPs.[223, 224] For the preparation of a suitable delivery system for implantation in periodontal defects in the baboon, aliquots of insoluble collagenous matrix, the inactive residue obtained after dissociative extraction of the matrix with 4 M guanidine-hydrochloride, were reconstituted with 250 µg of BMP fractions per pellet and lyophilised. This preparation is composed predominantly of BMP-2, BMP-3 (osteogenin), and OP-1 (BMP-7). Pellets were then implanted into large furcation defects surgically created in the 1st and 2nd mandibular molars of 4 baboons.[205] The depth of each furcation defect extended for at least 10 to 12 mm in the bucco-lingual direction as measured from the buccal entrance of the exposed furcations of the 1st and 2nd molars. Pellets of collagenous matrix without BMPs were implanted without BMPs as control. For further

elucidation of the histological events during repair and regeneration, specimen blocks, harvested on day 60, were embedded, undecalcified, in resin. Serial sections of 7-μm thick, including root dentin and associated periodontal tissues, were cut throughout the entire bucco-lingual extension of each treated defect. Histological analysis showed that BMPs, in conjunction with the collagenous matrix, induced cementum, periodontal ligament, and alveolar bone regeneration. The preparation and analysis of undecalcified sections cut at 7-μm were novel, and permit one to differentiate between newly formed and mineralized bone from the as-yet-unmineralized bone or osteoid, which stains orange-red by Goldner's method.[205] More importantly, foci of nascent mineralization could be seen within newly deposited cementoid (yet to be mineralized) in the coronal regions of the defects. The coronal extension of new attachment formation and of alveolar bone regeneration on both mesial and distal roots facing the exposed furcations was significantly greater in BMP-treated specimens when compared with furcation defects implanted with collagenous matrix without BMPs.[205] In addition, morphometric analysis of the furcation areas showed that mineralized bone and osteoid volumes were significantly greater in BMP-treated specimens compared with control. Interestingly, cartilage was never observed. Shorter time periods, however, are required for a chondrogenic phase during initial tissue morphogenesis to be completely ruled out after application of BMPs in the primate.[189]

Using the baboon model described above, Ripamonti *et al.*[205] treated furcation defects, prepared in the first and second mandibular molars, with 100 and 500 μg of recombinant hOP-1 (BMP-7) with collagenous matrix carrier (approximately 200 mg of carrier matrix was used per furcation defect).[198] After 60 days of healing, histological and histometric

analysis on serial, undecalcified sections cut at 7-μm showed substantial cementogenesis on the exposed dentin of furcations treated with both doses of hOP-1. Formation and insertion of Sharpey's fibers into newly formed cementum were also observed. Since hOP-1 in conjunction with the collagenous matrix induces bone formation in bone defects of the cranial and appendicular skeleton of the primate,[225, 226] it is likely that the expression of specific cell phenotypes by hOP-1 is regulated, in part, by the microenvironment and the extracellular matrix substrata.[198] Thus, in a periodontal defect, the presence of exposed dentin may preferentially modulate the expression of the cementogenic phenotype on readily available cell populations (pre-cementoblasts and their progenitors) from the periodontal ligament space.[189]

Sigurdsson *et al.*[208] suggest that placement of a rhBMP-2-containing device not only regenerates original alveolar bone height, but additional periodontal attachment apparatus as well. He used an unique model that involved creating supra-alveolar periodontal defects, removing the coronal portion of the tooth crown, and submerging the tooth beneath the gingival flap. Histologic observations revealed significant bone and cementum regeneration for defects treated with recombinant-human BMP-2 as compared with those defects treated with a control vehicle.[201]

The histological demonstration of new attachment formation (new cementum with inserted functionally oriented connective tissue fibers on a root surface that has been deprived of its periodontal ligament) in human material is crucial for our understanding of the healing potential of the periodontal tissues[94, 217, 227] Histological examination of human biopsies has shown that partially purified BMPs, isolated from human bone matrix in conjunction

with allogeneic lyophilised DBM, enhance new connective tissue attachment and alveolar bone regeneration in a root-submerged environment.[228] However, in a non-submerged environment, BMP fractions did not significantly increase periodontal regeneration when compared with controls of DBM.[228] The clinical significance of these histological findings is, at the moment, difficult to evaluate, and it will require controlled clinical trials using recombinant human BMPs produced by DNA recombinant technology, as opposed to bone-derived mammalian BMPs.[205]

rhBMP-2 used in an absorbable collagen sponge device[206] was proven to be safe and successful in the preservation of the alveolar ridge after tooth extraction or augmentation of localized defects. Bone regeneration was evident in all alveolar defects filled with the device. The same device used for maxillary sinus floor augmentation showed a significant bone growth, no immunologic or adverse effects, and no clinically significant changes in complete blood count, blood chemistry, or urinary analyses.[207]

1.7.8. Concluding Remarks

An important question is whether the presence of multiple form of BMPs has a therapeutic significance. Recombinant hOP-1, rhBMP-2, and rhBMP-4 are equally capable, after a single application, of induction of bone as well as reparative dentinogenesis in post-natal animal models,[194, 196, 229, 230] raising important questions on the biological significance of this apparent redundancy.[205] The finding that doses of 100 to 500 μg of hOP-1 (BMP-7) per gram of carrier matrix preferentially induced cementogenesis on denuded root surfaces indicates a specific function of recombinant hOP-1 during repair and regeneration of periodontal tissues.[198] Future research must focus on optimal doses and molecular

combinations, developing a structure-activity relationship among the members of the BMP family.[189]

It has been implied that other BMPs could augment the osteogenic capability of BMP-2, either through interaction with the same target cell or by acting on completely different cell populations during the complex process of bone formation.[192] This suggests a synergistic effect between BMPs which could potentiate bone formation.

In considering the number of BMPs isolated thus far, it may also be reasonable to assume that they are synergistic with each other. As multiple forms of natural BMPs are usually isolated from decalcified bone matrix, this combination when re-implanted to induce bone formation may allow for a more potent effect than using any single recombinant BMP.[231]

More than 50 years have passed since the first experiments were conducted which led to the theory of extractable substances from bone being able to induce new bone formation. The subsequent discovery and purification of BMPs have answered questions concerning not only the mysteries of heterotopic bone induction, but also that of normal skeletal regeneration and repair.[49]

To date, an investigation regarding the effect of 2 dosages in a combination of BMPs in a collagen carrier device has been undertaken but not yet published. (Ripamonti, personal communication).

CHAPTER 2: PROPOSED HUMAN EXPERIMENTAL PROCEDURE

2.1. MATERIALS AND METHODS

This research protocol is to be submitted to the Human Ethics Committees of the relevant institutions before initiating the clinical trial. A multi-centre study consisting of approximately 20 patients per centre will be conducted. Patients may be chosen from well-constructed periodontal treatment programmes. As an inducement patients will receive the equivalent of a full fee waiver for one quadrant of osseous surgery and the bone regenerative devices (BRD) if they participate in the study. All patients will have to meet the selection criteria below.

2.2. DEVICES TO BE USED IN THE STUDY

The devices to be implanted in the bony defects will consist of native bovine BMP-2, -3, and -7 added to human insoluble collagenous bone matrix (ICBM) as carrier.

2.2.1. Purification of BMPs

BMPs are purified from bovine bone matrix as described by Lyuten *et al.*[204] and Ripamonti *et al.*[223] Briefly, dehydrated diaphyseal bovine cortical bone (74 to 420 μm particle size) is demineralized in 0.5 N HCl and dissociatively extracted in 6 M urea, 50 mM Tris-HCl, pH 7.4. The protein extract is loaded sequentially onto heparin-Sepharose and

hydroxyapatite-Ultrogel affinity and adsorption chromatography columns, washed and eluted.

The protein concentrate is then loaded onto Sephacryl S-200 HR (high resolution) gel filtration columns, equilibrated and eluted. Final purification is achieved by heparin-Sepharose affinity chromatography of protein fractions after Sephacryl S-200. Highly purified BMP is then concentrated and exchanged with 5 mM HCl, the liquid vehicle for the formulation of the BMP Bone Device (BBD).

2.2.2. Carrier Matrix

There is homology in the bone morphogenetic proteins from human, baboon, bovine and rat bone matrix.[235] Bone induction by intact bone matrix is, however, species-specific, and the apparent specificity of xenogeneic bone matrices is due to immunogenic and inhibitory components in the insoluble collagenous bone matrix.[222] For therapeutic applications in humans, the carrier matrix for the preparation of the BMPs is thus human ICBM.

Human ICBM, used as carrier for the native bovine BMPs, can be prepared as described in detail in reference.[235] Briefly, diaphyseal bone segments are washed extensively in cold sterile deionized water, defatted in two changes of absolute ethanol in the cold and dehydrated in ethyl ether. Cortical segments are pulverised and sieved to a discrete particle size of 74-420 µm, demineralized in 0.5 N HCl, washed twice in cold deionised water, dehydrated in two changes of absolute ethanol, and dried after a wash in ethyl ether. Aliquots of BMPs in 5 mM HCl are added to the carrier matrix (1 mg BMPs per gm of matrix) in sterile glass vials, freeze-dried and subjected to gamma irradiation.

2.2.3. Inclusion Criteria[234]

1. Patients able and willing to give informed consent and follow a programme of plaque control (Appendix X).
2. Patients able and willing to be submitted to multiple follow-up examinations.
3. Males and females above the age of 21 years.
4. Females of child bearing age on an adequate hormonal contraceptive regimen. Unless post-menopausal, or surgically sterilised, these patients will have to produce a negative pregnancy test performed 3 days before implanting the device. The contraceptive regimen will be prescribed or supervised by a medical practitioner.
5. Scaling and root planing of all quadrants completed at least 8 weeks before surgery.
6. Patients demonstrating a satisfactory plaque control before surgical therapy. Plaque control record (O'Leary)[232] must be less than 20%; gingival index (Ainamo and Bay)[233] must be less than 10%.
7. At least one tooth in a contralateral quadrant with similar angular or class II furcation defect.
8. Involved teeth being vital and free of deep caries.
9. Angular defects $\geq$ 3 mm in depth as assessed by radiographic evaluation with a paralleling aiming device (Rinn apparatus).
10. In interproximal defects, radiographic evidence of 30% to 70% bone loss around study teeth. For furcation defects, radiographic evidence of furcation involvement at baseline.

2.2.4. Exclusion Criteria[234]

1. Subjects' inability or unwillingness to give informed consent.
2. Smoking or other use of tobacco products.
3. If Alcohol or drug abuse.
4. Non-vital experimental teeth.
5. Grade III mobility of teeth.
6. Female, positive pregnancy test 3 days before surgery or not practising adequate contraception.
7. Patients taking medicaments which are known to interfere with wound healing such as cytostatic agents, endocrine therapy influencing bone healing, immuno-modulating agents, or immuno-suppressive agents, or who have received such drugs within 4 weeks before application of the BRD.
8. Current or previous history of systemic diseases known to interfere with adequate wound healing such as chronic liver failure, chronic renal failure, diabetes mellitus, vascular diseases, malnutrition, AIDS and any other systemic infection.
9. Evidence or previous history of significant and/or unstable cardiovascular, pulmonary, gastrointestinal, haematological, or endocrine disease or other disorder which would impact on the clinical management of the patient.
10. Current or previous history of cancer (with the exception of squamous cell and basal cell carcinomas) within 5 years.
11. Periodontal defects of less than 3 mm in depth as assessed by radiographic evaluation.

12. Unacceptable plaque control, i.e., a PI > 20% and a BI > 10%.
13. Patients with refractory periodontitis.
14. Patients who have received an investigational drug or participated in a research study within 30 days before the first application of study device.

All patients selected for the study will undergo a complete dental examination for initial documentation including, but not limited to, a medical anamnesis, dental anamnesis, complete periodontal charting, a periapical radiographic status and clinical photographs. A comprehensive treatment plan will be presented to the patient which include his periodontal status and recommended treatment. The patient would be further informed that he has a specific type of bone defect in a specific location about which a study is currently conducted. The study will be an evaluation of treatment results as determined by direct measurement of the specific areas at a re-entry procedure six months later.

2.2.5. Description of surgical procedures

Each subject will present matching contralateral furcation or cuneiform bony defects. All participants will receive plaque control instructions, scaling and root planing before surgery.

a) Pre-operative measurements:
   1) Gingival recession as measured from the cemento-enamel junction (CEJ).
   2) Attachment level as measured from the CEJ.
   3) Pocket depth.

b) Standardised peri-apical radiographs, taken at the initial examination, will be used as a baseline to compare the dimension of the bony defects with the 6th and 12th months post-operative radiographic evaluation.

c) Mucoperiosteal flaps will be raised buccally and lingually (or palatally) to gain access for soft tissue debridement and root planing.

d) Measurements at the time of surgery:

1) Cuneiform defects

- Distance between the CEJ and the base of the bony defect.
- Distance between the CEJ and the coronal margin of the defect.

2) Furcation defects

- Distance between the roof of the furcation and the most apical extent of the bony defect. (See figure 1)
- Distance between the most external surface of the roots and the horizontal extent of the defect as measured with two periodontal probes. The first probe is placed horizontally on the external radicular surfaces at the apical base of the defect, and the second probe is also placed horizontally at the base of the defect, but perpendicular to the first. (See figure 2)

e) If the bony defect is corticalized at the time of surgery, the cortex will be perforated with a small round bur to open the marrow spaces to enhance

migration of osteo-progenitor cells and undifferentiated cells into the defect and the device.

f) After implantation of the device into the bony defects, the mucoperiosteal flaps will be replaced at their original level and sutured.

g) Patients will be examined one week later to assess healing and remove any remaining sutures. For the first month, the patients will be seen weekly to measure the PI. They will then be assessed monthly for the first trimester. Thereafter, recall appointments will be scheduled every 2 months. At each recall session, the PI will be recorded and scaling and polishing will be performed if needed. Plaque control techniques will be re-inforced when necessary. Probing depth will only be re-evaluated 2 months after surgery, and at subsequent appointments, the PI, BI, and probing depth (PD) will be measured.

h) Post-operative measurements:

1) Level of gingival margin.

2) Attachment level.

3) Sulcular depth.

i) Measurements at re-entry

1) Cuneiform defects

- Distance between the CEJ and base of remaining osseous defect or regenerated area.

- Distance between the CEJ and the crestal margin of the defect.

2) Furcation defects

- Distance between the roof of the furcation and the most apical extent of the bony defect. (See figure 1)
- Distance between the most external surface of the roots and the horizontal extent of the defect as measured with two periodontal probes. The first probe is placed horizontally on the external radicular surfaces at the apical base of the defect, and the second probe is also placed horizontally at the base of the defect, but perpendicular to the first. (See figure 2)

j) Six months after surgery, a peri-apical radiograph of the operated region will be taken and, upon radiographic and clinical evidence of bone regeneration, a trephine biopsy will be taken at the site of a previous defect. The site of the biopsy will then be implanted with the same device used previously. However, no biopsy will be taken if the site is not suitable, as in very narrow furcation areas due to close proximity of the roots, or close proximity of adjacent roots between the teeth where a vertical bony defect was treated. Should it be found that the regeneration was incomplete, the same material will be re-implanted in the original area.

k) The biopsy specimens will be processed to obtain undecalcified sections and evaluated histologically to assess the percentage of newly mineralised bone, osteoid and fibro-vascular spaces in the regenerated area. (See reference 236)

l) Controls will be based on historical knowledge of expected regeneration. (See references 23, 36, 47, 48, 205 and 228)

CHAPTER 3: CONCLUSION

For decades, researchers and clinicians have applied different methods to restore the functional components of the periodontium. Presently, satisfactory and predictable periodontal regeneration results from the application of affordable native proteins that initiate and enhance the biological processes necessary to achieve true regeneration to its full potential. The technical evolution of periodontal augmentation, and eventually regeneration, has invested researchers with a better understanding of the very intricate processes involved in bone healing and has spurred the development of treatment options to what they currently are.

However, in the field of growth and regeneration, there is still a lot of unknown territories which are currently being explored or need to be investigated in the future. This dissertation provides a brief overview of a very wide field of past experiments, as well as current methods and proposes the implantation of a BMP-device in human periodontal osseous defects.

The realisation of this proposed study will undoubtedly result in new knowledge that may contribute to reach the ultimate goal in periodontal therapy. The wider implication of this study, resides in dental implantology and maxillo-facial surgery.

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APPENDIX A

INFORMED CONSENT FORM

I, Mr./Mrs._____,

born _____,

agree to the surgical implantation of a bone regenerative device (BRD).

- I have been informed that there is no guarantee that the BRD wilI remain stable over time. I understand that in a certain small percentage of cases, such implants may be resorbed after various periods of time.
- In addition, I have been informed of the alternative treatments that would be possible, and of the complications that can occur with the implant treatment as planned.
- I have been assured that only accepted procedures will be used. I leave my treating dentist the choice of implant type.
- I am aware that it may be necessary to extent or alter the planned procedures during the operation. All of my questions have been answered. An explanation of all details has been provided to my satisfaction. I understand that I can withdraw my consent at any time.
- I consent to the proposed treatment as well as to any necessary extensions or charges. I attest that I have made known in the medical /dental questionnaire any and all diseases and disorders known to me.
- Finally, I have been informed that the success of the treatment procedure depends largely on regular follow-up appointments.

Location:_____ Date_____

_____ _____

Signature of the patient Signature of the attending dentist

_____ _____

[Witness] [Witness]

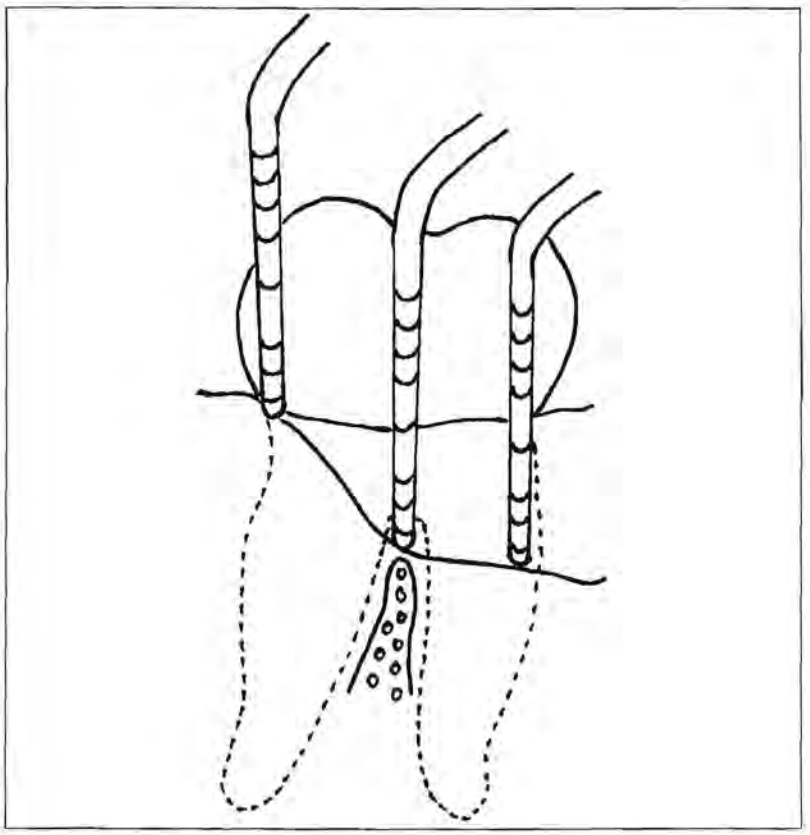

Figure 1:
Measurement of the distance between the roof of the furcation and the most apical extent of the bony defect.

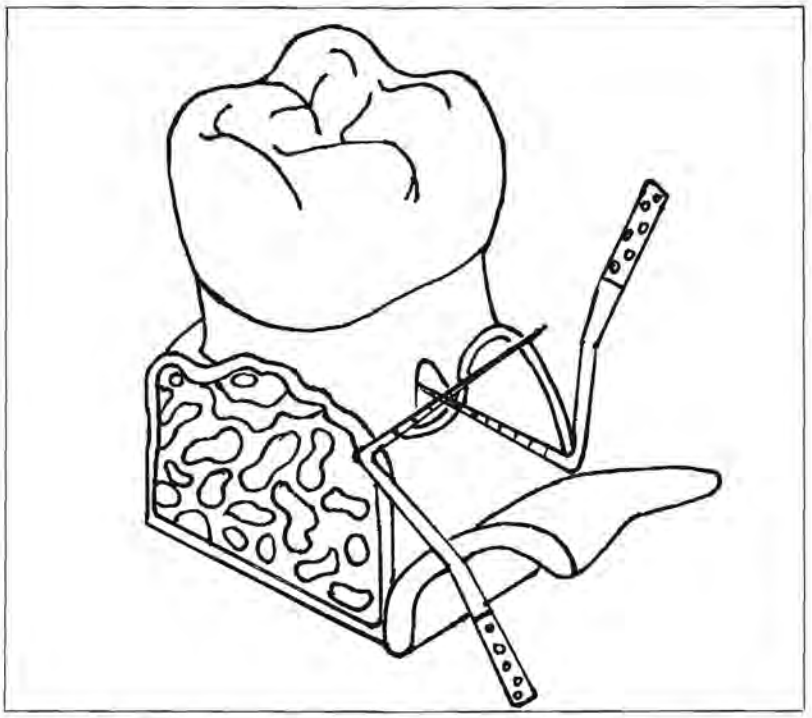

Figure 2:
Measurement of the distance between the most external surface of the roots and the horizontal extent of the defect as measured with two periodontal probes.