MANAGEMENT OF EXPERIMENTAL PERI-IMPLANTITIS AROUND POROUS-SURFACED IMPLANTS IN DOG

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science Graduate Department of Dentistry University of Toronto

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Irina Vitcu, Master of Science
Graduate Department of Dentistry, University of Toronto, 2000.

Abstract
The purpose of this study was to compare two approaches for the management of experimental peri-implantitis induced by cotton ligatures placed around porous-surfaced dental implants. Thirty implants were placed in 5 beagle dogs, 3 fixtures on each side of mandible, 4 months after extraction of the mandibular premolars and first molars. After re-entry and abutment connection, a cotton ligature was placed around the neck of each implant and forced apically to disrupt the soft tissue seal of the peri-implant space. Oral hygiene was withdrawn and administration of a soft diet allowed gross plaque accumulation. Periodic radiographic and microbiologic exams were performed. After 6-8 weeks the ligatures were removed and regular hygiene re-instituted. Four weeks later and after commencement of systemic antibiotics, the “rescue” surgical procedures were performed. These were based on GBR principles and done using a split-mouth model design. On one side of the jaw Dynagraft® Putty was used as a graft while on the other side, the same graft material was used along with Capset™ as a barrier.

Results from microbiologic, radiographic, clinical and microscopic assessments revealed that: (i) Porphyromonas gingivalis was detected at all peri-implantitis sites as the disease progressed and at the time of rescue surgery, up to 3 mm of the porous surface was no longer submerged in bone because of the bone loss that occurred; (ii) Assessment of prepared sections using BSEM and statistical treatment of the data indicated that both treatment approaches i.e., Dynagraft + Capset and Dynagraft only, were effective in terms of inducing new bone formation and allowing for re-osseointegration to varying degrees; (iii) Dynagraft + Capset treatment group had significantly better values for the regained bone height and bone ingrowth fraction for defect segment, parameters that reflect the level of re-osseointegration induced by the treatment.
First and foremost, I would like to express my gratitude and appreciation to my supervisors, Dr. Douglas Deporter and Dr. Robert Pilliar. Their support and encouragements for the past two years gave me the confidence and made me live an incredibly rich and rewarding professional experience. I see now with passionate eyes the results of my work and I am very proud to have been part of such an elite group of dental implantology researchers. I have to mention here the constant effort and support that I have received from them in the editing of this thesis, for it would not have been the same without their input and ideas.

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Last, but not least, I will be always grateful to my husband Adrian, for his unconditional love and support and for being the first to believe in me and my potential.

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List of abbreviations

ANCOVA: analysis of covariance
BSEM: backscattered scanning electron microscopy
FDDBA: freeze-dried demineralized bone allograft
GBR: guided bone regeneration
HA: hydroxylapatite
Pg: Porphyromonas gingivalis
SCR: smooth collar region
I. Literature review

1) Endosseous dental implants

Osseointegration

The use of endosseous dental implants in the routine management of partial and complete edentulism is fast becoming commonplace with implants replacing the conventional dental prosthetic appliances. Used appropriately for a particular application, most current implant systems can be successful. After implantation, the preferred response of the host bone towards the implant is osseointegration. Below is discussed briefly the current knowledge on the implant osseointegration process, pre-requisites for dental implant osseointegration and success criteria.

Osseointegration of dental implants has been defined by many authors. Albrektsson et al (1981) defined osseointegration as "direct functional and structural connection between living bone and the surface of a load bearing implant". Further, Zarb and Albrektsson (1991) clinically described osseointegration as “a process in which a clinically asymptomatic rigid fixation of alloplastic material is achieved and maintained in bone during functional loading”. The osseointegration of dental implants is a complex process, that is only partially understand despite a number of methodical investigations and reviews reported in the literature (Cooper et al, 1998; Davies, 1998; Masuda et al, 1998 and others).

Davies (1998) subdivides the mechanisms by which endosseous implants become integrated into three separate phenomena; osteoconduction, *de novo* bone formation and bone remodeling. Osteoconduction relies on the migration of differentiating osteogenic cells to the implant surface through the fibrin network that forms during blood clot resolution. Some of these cells will reach the mature status before reaching the implant surface, while others will reach the same surface undifferentiated and then will differentiate, starting to synthesize bone matrix right on the surface of the implant. It is
clear that the implant design (i.e. surface topography) will influence osteoconduction by affecting the maintenance of anchorage of the temporary scaffold (i.e. fibrin network). It can be predicted that a roughened surface would promote osteoconduction by increasing the area on which fibrin can attach and also providing features with which the fibrin can become entangled. In addition, surface chemistry can influence the absorption and retention of macromolecules (Davies, 1998). De novo bone formation can be described as a cascade of four events (similar for bone formation at an implant surface or old bone surface) briefly described as: (i) secretion of a collagen-free matrix by the differentiating osteogenic cells that provides (ii) nucleation centers for calcium phosphate mineralization. Crystal growth then takes place concomitant with (iii) initiation of collagen fiber assembly and finally, (iv) the calcification of these fibers takes place. Bone remodeling has importance for the long-term stability of the implant. The phenomenon takes place in both cortical and trabecular bone, and comprises, at discrete sites, de novo bone formation.

Albrektsson et al (1981) listed the following prerequisites for implant osseointegration:

1) Implant material: it is now agreed that titanium (commercially pure or as alloy with aluminum and vanadium) is the material of choice for dental implants. The preference is based on its resistance to corrosion, its tolerability by the host tissues and its mechanical properties.
2) Implant design: first the dental implant was cylindrical and threaded; currently implants are formed in several acceptable shapes
3) Implant surface topography: while at the beginning, only machined implants were recommended, currently machined, shot-blasted, plasma-sprayed, acid-etched, and porous-surfaced implants are considered acceptable
4) Status of the bone: unresorbed crestal bone is preferred. Currently, a number of techniques for bone augmentation are at hand for clinicians. The status of the bone is dependent on endogenous systemic factors (age, genetics, general health [Baxter & Fattore, 1993] and smoking habits) and local factors such as anatomical location of the implants (Bryant, 1998; Sennerby, 1998).
5) Surgical technique: the approach recommended initially and still preferred involved implant placement using as atraumatic a technique as possible, avoiding excessive pressure and bone overheating. For this purpose, suitable instruments are used concomitantly with continuous saline irrigation. The use of prophylactic antibiotics to reduce the risk of infections is recommended.

6) Post-implantation treatment: Appropriate implant loading conditions are recommended namely the application of limited if any loading forces, the timing of loading (i.e. subsequent to bone healing occurs) and the avoidance of significant relative movement of implant (Kohn, 1992; Szmukler-Moncler et al., 1998).

Cochran (1999) suggested these additional criteria for implant success; they are listed below (adapted after Schnitman & Shulman, 1979; Albrektsson et al., 1986; Smith & Zarb, 1989):

1) Less that 1 mm mobility of the implant relative to its host bone in any direction
2) Absence of peri-implant radiolucency
3) Crestal bone loss should not be greater than 1/3 of the implant/bone interface length and less than 0.2 mm annually after the first year of service. Bone loss should not reach the apical 1/3 of the implant. Non-standardized peri-apical radiographs should demonstrate less than 50% of the implant has bone loss
4) Provide functional service for 5 years in 75% to 85% of cases; 80% success after 10 years
5) Absence of persistent and/or irreversible signs/symptoms such as pain, neuropathies, paresthesia, violation of mandibular canal; absence of persistent soft tissues complications
6) Implant design should allow restoration satisfactory to the patient and dentist
7) No mechanical failure of the implant should occur
8) The implant should be surgically retrievable
Implant design variations

A number of dental implant systems with various designs, shapes, surface geometries and dimensions are currently commercially available, each one having its advantages and disadvantages.

Commercially pure titanium and titanium alloy (Ti6Al4V) and hydroxyapatite (Ca$_{10}$[PO$_4$]$_6$[OH]$_2$) are currently the major materials used for dental implants in North America (Smith, 1993), because of titanium’s corrosion resistance and biocompatibility and presumed bone bonding properties of hydroxyapatite. It is recognized that all materials degrade in some degree in the body. Briefly, they undergo corrosion, dissolution, hydrolytic decomposition and may generate particles through wear. Critical to acceptance or “biocompatibility” is the demonstration for specific applications of an acceptable rate of degradation, and acceptable response over the short- and long-term of degradation products.

There are two categories of endosseous dental implants discussed in the literature namely one stage (non-submerged) and two stage (submerged) implants. Although the fixtures placed in one-stage procedures have the advantage that no second surgery is needed for the subsequent and latter transgingival abutment connection, inadvertently micromotion might disturb the initial healing phase while possible contact of the peri-implant space with the oral environment might compromise the expected outcome.

Results from animal experimental studies are available for comparison of these two implant categories. Weber et al (1996) compared in a split-mouth design study one- vs. two-stage CP Ti plasma-sprayed implants with a smooth coronal portion intended to be in contact with soft tissues. They concluded that no significant differences were detected between the two placement techniques for the linear measurements from the top of the implant to mucosa border, top of the implant to the most coronal bone level in contact with the implant and the length of the connective tissue contact. When epithelial downgrowth and the probing attachment level were considered, the authors found
significant differences that favored the one-stage implant group. They found that the apical extension of the peri-implant epithelium was greater for the submerged group and also the probing attachment level was significantly lower for the same group. In this study the evaluation of the above listed parameters was done 4.5 months after the implantation.

A study by Abrahamsson et al (1999) failed to find any differences between submerged and non-submerged titanium implants with sandblasted surfaces. Nine months after the implantation the authors evaluated the height of the mucosa, the length of the junctional epithelium, the height of the connective tissue integration zone, the percent of bone to implant contact and the density of implant bone for the two groups and found them similar.

Ericsson et al (1996b) evaluated the performance of one- and two-stage Branemark implants placed in beagle dogs. Six months after implantation of the one-stage fixtures the animals were sacrificed and radiographic and histological evaluations demonstrated comparable results in terms of soft tissue adaptation and osseointegration. Although Ericsson’s results are comparable to those reported by Abrahamsson (1999) and in disaccord with those of Weber, all three studies failed to detect obvious disadvantages for the use of one-stage implant. The successful clinical use of implants placed using one-stage approach is documented for fixtures with Ti plasma-sprayed surfaces (Buser et al, 1991).

Levy et al (1996) compared the performance of porous-surfaced dental implants placed using one- or two-stage approach in beagle dogs. The evaluation was limited to parameters that characterize osseointegration 6 weeks after implantation. The histomorphometric analysis carried out revealed that bone-implant contact was greater for the submerged group, suggesting that bone healing may be delayed for the non-submerged fixtures. The findings are in disaccord with those reported by Abrahamsson et al (1999) that found comparable values for the percent of bone-to-implant contact in the submerged and non-submerged groups. The difference might be due to different time of
evaluation (9 months in Abrahamsson’s study and 6 weeks in Levy’s). Overall, Levy’s study demonstrated that in beagle dogs the porous-surfaced dental implants could be integrated in 6 weeks after placement using a non-submerged approach.

Topography and chemistry of the dental implant surface are elements that influence both, the initiation and maintenance of osseointegration. These dental implant surface characteristics are discussed in greater detail below in relation to the capacity of different surfaces to harbor dental plaque and microorganisms.

2) Recognized failure modes for dental implants

Two major causes of failure of endosseous dental implants are mechanical overload either due to premature loading and/or subsequent mechanical overload during function and infection caused by dental plaque or other microorganisms adhering to the implant surface (Ellen, 1998; Esposito et al, 1998a, b).

a) Mechanical overload

The desired response of host bone to dental implants following implantation is osseointegration. Also, the soft tissues that constitute a seal of the peri-implant space are expected to be closely adapted to the implant surface and healthy. After osseointegration, the fixtures might maintain this status and be confirmed as successful or their stability and functionality might be compromised, therefore causing implant failure.

Esposito et al (1998a) and Szmukler-Moncler et al (1998) reviewed the literature published on timing of loading and the effect of micromotion on the bone-dental implant interface and concluded that the early loading per se was not detrimental for osseointegration, but only when associated with poor bone quality and dependent on implant type. Only excessive micromotion (over 150 microns magnitude, the accepted level being 50-150 microns) was directly implicated in fibrous tissue encapsulation and prevention of osseointegration. Such huge displacement presumably influence cell
differentiation within the implant-tissue interface zone toward fibroblast pathway and fibrous tissue formation.

Failure can also result due to inadequate loading/overloading subsequent to the establishment of osseointegration (i.e., following successful healing of the implantation site). Briefly, the mechanism by which implants can fail due to excessive forces in this case can be summarized as follows: once the biomechanical load exceeds the load-bearing capacity of the host bone, microfracture will develop at the implant-bone interface zone. The normal bone remodeling can overcome the microdamage if microfractures accumulate at a slow pace. If not, the fixture will eventually fail due to loss of fixation (Carter, 1984).

Meffert (1993), Saadoun et al (1993) and Esposito et al (1998b, 1999) when reviewing the literature on overloading and increased rate of dental implant failure concluded that indeed, a relationship exists between the two.

Osseointegrated implants can fail due to excessive occlusal load as demonstrated by Isidor et al (1996) in a monkey experimental study. However, it would be difficult to demonstrate this in humans, as it is hard to quantify the actual biting forces supported by an implant (Cochran, 1996). To overcome this problem a number of two- and three-dimensional finite element analyses studies simulating the stress distribution in bone around dental implants have been undertaken in an attempt to help in understanding the issue.

Barbier et al (1998) found a strong correlation between the calculated stress distribution determined by axial and non-axial loading when compared to the remodeling phenomena in a comparative animal model. This study utilized IMZ implants placed in beagle dogs and supporting a fixed partial prosthesis and a cantilever fixed partial prosthesis. It was concluded that the highest bone remodeling events coincided with the regions of highest equivalent stress as determined by finite element analysis.
Meijer et al (1996) calculated the stress distribution around dental implants in human edentulous mandibles by means of a three-dimensional finite-element model of the anterior part of the jaw. The implants were connected by a bar or were solitary. Besides other observations, the investigators concluded that the extreme stresses in bone were always located around the necks of the implants.

From the prior studies it can be concluded that a certain amount of crestal bone loss occurs invariably. Adell et al (1981) studying threaded titanium implants: suggested that the mean crestal bone loss was less than 1.5 mm during the first year of function and 0.2 mm per year, thereafter the implant could be considered successful. This pattern of bone loss has been related to excessive stress concentration, as predicted by finite element analysis to develop at the bone-implant interface of the threaded designed implants (Clelland et al, 1990; Reiger et al. 1990a, b). The same trend was observed for cylindrical, press-fit designs with Ti or HA plasma-sprayed surfaces, and again, high stress concentration in the coronal regions were proposed as the reason for crestal bone loss in this case (Holmes et al, 1992). In some cases the crestal bone resorbed to a level that exposed the irregular surface of the plasma-sprayed implants thereby making them more susceptible to plaque accumulation and failure because of peri-implantitis (see below).

If the bone into which implants are placed is of poor quality due to anatomical location or to diverse systemic illnesses that affect bones, higher failure rates can be expected due to excessive or even normal functional forces. However, recent investigations failed to detect any relationship between high failure rate and bone of poor quality (Balshi et al, 1997); the authors claimed the subjectivity and irreproducibility of the bone quality measurement.

Parafunctions (i.e. bruxism and clenching) are also implicated in inducing marginal bone loss around implants. The mechanisms causing this are basically similar to overloading, as these actions generate increased and/or faultily oriented forces. A consensus exists that excessive loading or excessive stresses may induce bone loss (World Workshop in
Periodontics, 1996). Lindquist et al (1988) reported increased marginal bone loss in patients prone to clenching, although no information was disclosed about how the measurements were made. Excessive forces can determine marginal bone loss around implants as well. Quirynen et al (1992) observed a correlation between increased marginal bone loss around implants and occlusal overload. Naert et al (1992) observed that increased vertical dimensions in partially dentate patients treated with prostheses supported by implants led to implant failure with no signs of inflammation (i.e., peri-implant mucositis).

**Stress-shielding resulting in peri-implant pocket formation**

As noted above crestal bone loss due to mechanical effects may contribute to development of a scenario promoting peri-implant infection. A further complication that may influence bacterial colony development in the peri-implant region is the possibility of crestal bone loss due to stress-shielding, that will not necessary induce implant failure but can initiate the circumstances for this to occur.

Stress-shielding related to dental implants is a phenomenon that take place in the crestal bone adjacent to machined regions of implants and is caused by local under stressing as opposed to overloading as discussed previously.

It is hypothesized (Pilliar et al, 1991) that because of a lack of effective mechanical coupling between a coronal smooth titanium surface regions of an implant that is well fixed to surrounding bone apically, less effective tensile and shear force transfer occurs, between the implant and the host bone. This leads to under stressing and disuse atrophy of crestal bone. This was demonstrated in experimental studies in beagle dogs, by Pilliar et al (1991) and Al Sayyed et al (1994) studies that have used the porous-surfaced dental implant (i.e., with a machined collar at its most coronal part and with the remaining portion of its length porous-surfaced by a process of sintering). In the study by Pilliar et al (1991) the crestal bone loss was compared around fully porous-coated, partially porous-coated (titanium alloy) and machined threaded (commercially pure titanium)
implants over a 77-week functional period using radiography during function and histological sections after animal sacrifice. The study demonstrated that some crestal bone loss occurred in the first 20 weeks for the threaded implants and in the first 54 weeks for the partially porous-coated ones, while for the fully porous-coated specimens assessed after the same period of time, no significant bone loss was observed in relation to the coronal region. The study concluded that the crestal bone would be maintained when effective coupling between the bone and the implant surface occurred, but bone loss will happen next to the smooth implant region. The machined porous-to-porous surface junction for partially coated specimens represented the limit of their crestal bone loss.

This conclusion was supported by Vaillancourt et al (1996) who conducted a two-dimensional finite element analysis and concluded that for partially porous-surfaced dental implants (surface obtained by sintering), the observed crestal bone loss is the result of the lower stresses acting around the machined collar region of the implant, causing disuse atrophy.

In support of the findings by Pilliar, Hansson (1999) used a three-dimensional and axisymmetric finite element analysis to determine the stress distribution around an axially loaded dental implant with a smooth coronal segment and a dental implant provided with retention elements all the way up to the top of the implant. The author concluded that these retentive elements improved the capacity of the implant to carry axial loads reducing the disuse atrophy of the marginal bone. Thus, from a biomechanical viewpoint it appears to be advantageous to provide the neck of screw-shaped implants with retention elements. It is furthermore suggested that retention elements at the implant neck will counteract marginal bone resorption, preventing the exposure of the surfaces meant to promote osseointegration and their contact with the oral environment, decreasing the chances for plaque accumulation and further development of peri-implantitis.
b) Peri-implant infection

Although a combined etiology i.e., mechanical and infectious, for failure of dental implants cannot be excluded, microbial flora of implants affected by mechanical failures is consistent with flora that surrounds healthy peri-implant sites (Rosemberg et al, 1991). The implication of a microbial factor contributing to the failure of dental implants, usually referred to as peri-implantitis, is well documented. In peri-implantitis an imbalance of host-parasite equilibrium manifests itself in a series of inflammatory changes leading to two distinct conditions: peri-implant mucositis i.e., a lesion of the superficial soft tissues and peri-implantitis, a lesion that involves the deeper soft tissues as well as the marginal peri-implant bone (Tonetti & Schmid, 1994).

Indeed, it has been experimentally demonstrated in humans and animals, that when oral hygiene procedures are discontinued plaque accumulation leads to gingivitis in 10-21 days (Leonhardt et al, 1992; Carranza & Newman, 1996) or peri-implant mucositis. Further plaque accumulation and maturation will finally result in crestal bone loss around natural teeth or implants.

Clinically the mucosa around implants affected by peri-implantitis displays recession, bleeding, inflammation and suppuration. By radiographic examination an area of vertical and/or horizontal crestal bone loss can be described. By histological assessment, in humans, the peri-implant lesions are characterized by an increased infiltrate of plasma cells and mononuclear cells (lymphocytes, monocytes, macrophages). Animal studies in which peri-implantitis was induced by means of ligatures have provided additional information on this subject. Osteoclasts were observed at the bone surfaces facing the inflammatory infiltrate (Esposito et al, 1998b).

Microbiologically, peri-implantitis has been associated with the presence of spirochetes, motile rods and Gram-negative obligate or facultative anaerobic bacteria, flora that differs from that associated with healthy sites, mainly consisting of Gram-positive,
aerobic microorganisms. As the peri-implant tissues are "invaded" by periopathogens, bacterial antigens, irritants, toxic products and harmful enzymes are released and have a potential negative effect on connective tissue causing complement activation, tissue disruption, cell membrane damage, cytotoxicity and bone resorption. The microbial adherence on oral structures, i.e., teeth and implants, depends on the characteristics of those structures, i.e., surface topography and surface free energy. This will be discussed below.

It is a scientifically supported fact that good oral hygiene can prevent plaque accumulation and maintain a healthy status of the gingival, periodontal and peri-implant tissues in humans and animals. Tooth brushing with water and pumice and rinses with antibacterial substances as Chlorhexidine 0.12% reinstated a healthy gingival condition as demonstrated in animal studies where marked gingival inflammation had been induced by ligatures, soft diet and hygiene procedure cessation (Leonhardt et al, 1992; Grunder et al, 1993; Hurzeler et al, 1995; Ericsson et al, 1996a; Persson et al. 1996). Several authors advocated the use of Chlorhexidine 0.12% as rinsing solutions or crevicular irrigations in patients with peri-implant mucositis and reported reduction of gingival inflammation and bleeding (Lavigne et al. 1994; Ciancio et al, 1995).

When the preventive action fails to maintain healthy peri-implant tissues and peri-implantitis develops impairing the functionality of the implants and inducing patient discomfort, surgical procedures are available for the treatment of the condition. Again, this is discussed in detail below.

**Biofilms on dental implants**

Without the initial attachment to implant surfaces by bacteria, subsequent polymicrobial accumulation and colonization leading to peri-implant disease cannot occur. The majority of bacteria that colonize humans display sharp tissue tropism. Particularly, the mouth has a variety of features that facilitates bacterial adhesion. It is reasonable to assume that
microorganisms can also easily colonize the titanium or hydroxyapatite surfaces of dental implants, the sequence of colonization being similar to that described above for teeth.

Such bacterial adhesion occurs in four phases (Quirynen et al., 1994c): transport to the surface, initial adhesion with a reversible and irreversible stage, attachment by specific interactions and, finally, colonization. During this process the roughness and the free energy of the surfaces play key roles.

Steinberg et al. (1995) and Kohavi et al. (1997) demonstrated that human albumin and α-amylase are salivary proteins that adhere to titanium powders as well to enamel powders or enamel and dentin hard surfaces. Zeng et al. (1999) advocated that surface chemical composition and roughness might influence the kinetics of protein adsorption and indeed, they demonstrated experimentally that calcium phosphate surfaces adsorbed a greater amount of bovine serum albumin than titanium ones. Serro et al. (1997; 1999) studied the absorption of bovine serum albumin on titanium oxide surfaces (TiO₂) and concluded that the absorbed amount is always lower in the presence of calcium and phosphate ions, suggesting that this is related to the fact that TiO₂ surfaces become hydrophilic when in contact with solutions containing these ions. All the above studies demonstrated that implant surfaces can adsorb proteins from saliva and this might help further bacterial adherence and colonization. Indeed, Nesbitt et al. (1982) demonstrated that cell surfaces of S. sanguis have hydrophobic properties that are more likely to help them to adhere via hydrophobic bonds to salivary proteins usually found on the oral hard surfaces. Furthermore, Pratt-Terpstra (1987) conducted an investigation showing that the number of oral streptococci (S. mitis, S. sanguis and S. mutans) adhering to a surface of fluoroethylenepropylene copolymer, cellulose acetate and glass coated with bovine serum albumin is less than those that adhere to the same, bare, uncoated substratum.

Steinberg et al. (1998) established that the rate of adhesion in vitro of P. gingivalis and A. viscosus to titanium or titanium-alloy surfaces is reduced by first coating them with salivary albumin, a condition that would automatically occur in vivo. Thus, there may be natural inhibitory mechanisms that discourage some bacteria from adhering to dental.
implant surfaces. The author had two possible explanations for his observations; firstly, that salivary albumins might act by masking the electrical charges of the two abutting surfaces (bacterial and metal), or secondly, that they might interfere with the hydrophobic-based adhesions of bacteria. However, in vivo it is less likely that adherence of \textit{P. gingivalis} and \textit{A. viscosus} would occur directly to implant surface as it is suggested in this study, but to the first colonizers that are mainly \textit{Streptococcus} species.

Ichikawa et al (1999) observed that in vitro adherence of \textit{Streptococcus constellatus} (microorganism that can be isolated from peri-implantitis sites) is greater on HA-coated surfaces than on titanium ones, and further increases when the HA surfaces are etched with 5\% hydrofluoric acid for 10 to 50 seconds (in this study, surfaces roughness is not reported). Their findings are possibly explained by the fact that calcium phosphate surfaces adsorbed a greater amount of serum albumin than titanium ones, as demonstrated by Zeng et al (1999), validating the fact that microorganism’s adhesion on diverse implant materials is highly dependent on adhesion of salivary or serum proteins.

Surface free energy (reflected in wettability) needs to be considered when discussing implant surface characteristics because it has been reported to have an important influence on plaque accumulation and bacterial adhesion in the supragingival area. Quirynen et al (1989), for example, demonstrated in vivo in humans that the higher the surface free energy of a material, the more plaque will accumulate on it. Materials studied included fluorethylene propylene copolymer (FEP), parafilm, cellulose acetate and enamel. The respective surface free energies were 20, 26, 57, and 88 erg/cm². Furthermore, Quirynen et al (1990) found a proportional relationship between the quantity of plaque that accumulates on a surface with high free energy and which is also rough (Ra 2.2 \textmu m). They compared fluorethylene propylene, cellulose acetate and enamel with surface free energy of 20, 58, and 88 erg/cm², respectively and a roughness of Ra=+/- 0.1 \mu m to Ra=+/- 2.2 \mu m.

Quirynen (1994a) investigated by differential phase-contrast microscopy and DNA-probe analysis the influence of the surface roughness and free energy on suprag- and subgingival
plaque biology in patients with functional prostheses supported by endosseous dental implants. Two different types of abutments were used in the study: titanium (roughened by sandblasting, average Ra=0.81 microns as measured with Perthometer, a profilometer that performs profilometric surfaces analysis, surface free energy 80 erg/cm², as measured by the means of sessile drop method) and titanium coated with fluorethylenepropylene copolymer, (surface roughness Ra=0.82 microns, surface free energy 23erg/cm²). The test was conducted for 3 months while the patients performed habitual hygienic procedures. The authors demonstrated that on surfaces of equal roughness the supragingival plaque differed when the two abutment types were considered. Titanium abutments (with a higher surface free energy) harbored a lower concentration of coccoid cells, which can be interpreted as more mature plaque, while the FEP-coated titanium abutments had a retarded plaque maturation, more coccoid cells, which can be explained by a low binding force between bacteria and low energy surfaces. Subgingivally, the differences were not obvious, the authors' explanation being that the modified environment would allow bacteria to adhere to either hard surface or to the pocket epithelium. Quirynen's study (1994a) demonstrated that, in the case of an implant, surface free energy plays a role in bacterial adherence and suggested that by controlling this surface feature, (i.e. lowering the surface free energy), the plaque adherence and maturation would be minimized.

Adherence of bacterial plaque to a dental implant also is strongly influenced by the implant's surface topography, “rough” surfaces harboring an increased number of organisms (Wu-Yuan et al, 1995; Drake et al, 1999). This topic will be discussed in detail below.

The microbial flora associated with healthy and failing endosseous dental implants

On natural teeth, the first step in the development of supragingival microbial dental plaque is the formation of an acquired pellicle, and this occurs generally in less than an hour following professional tooth cleaning. This pellicle subsequently becomes populated with microorganisms by providing specific receptors such as bacterial cell fragments, proline-rich proteins, alpha-amylase which are recognized by the early colonizers.
Streptococcus spp. (S. sanguis, S. oralis) are the first bacteria to adhere, which then facilitate the adherence of Actinomyces spp. (A. naeslundi, A. israelii), Capnocytophaga spp., Prevotella spp., all within the first 2-8 hours of pellicle formation.

The link between early and late colonizers is mainly represented by Fusobacterium nucleatum. From the group of the late colonizers, Actinobacillus Actinomycetemcomitans, Prevotella intermedia, Treponema spp., Porphyromonas gingivalis are of interest because they are known to be associated with periodontal disease and peri-implantitis (Kolenbrander & London, 1993).

Subgingival plaque follows the accumulation of its supragingival counterpart, as the latter creates conditions for gingival inflammation and appropriate growth factors in the subgingival region. The adherence mechanisms of microorganisms forming subgingival plaque are similar to those described for supragingival plaque, but also include specific receptors for crevicular epithelial cells and specific bacterial adhesins. Steptococcus spp. and Fusobacterium are important early colonizers, which later will bind Gram-negative, anaerobic bacteria such as Actinomyces spp., Eikenella, Veillonella, among which, one will find the periopathogens as well (i.e., Porphyromonas gingivalis).

Once these bacteria are established in the sulcular or subgingival region, they will multiply if left undisturbed because they are no longer subject to the oral natural cleansing mechanisms of salivary flow, mechanical action of foods during mastication and tongue or cheeks during speech. As this happens, bacterial antigens, irritants, toxic products and harmful enzymes are released that lead to pathologic changes in the periodontal tissues including loss of bone. For example, P. gingivalis can produce enzymes such as collagenase, phospholipase A and proteases, metabolic products like mercaptans, hydrogen sulfide, propionate, butyrate and indole, endotoxins, exotoxins and epitheliotoxins. All these have potential effects on complement activation, connective tissue disruption, cell membrane damage, cytotoxicity and bone resorption.
Bacterial Plaque and Dental Implant Failure

Evidence suggests that in humans and animals similar flora may develop in relation to teeth or dental implants, and that the pathogens causing periodontal disease and those involved in peri-implantitis are similar (Slots et al., 1986; Ong et al., 1992; Gatewood et al., 1993; Silverstein et al., 1994; Hanisch et al., 1997; Mombelli & Lang, 1998). With successful implants, the organisms which colonize their exposed surfaces shortly after exposure to the oral environment (Koka et al., 1993; Leonhardt et al., 1993), remain relatively stable in composition over time and consist mainly of *Streptococcus* spp., *Capnocytophaga*, *Veillonella* spp., *Peptostreptococcus* spp. *Porphyromonas gingivalis* and *Bacteroides forsythus* can be sporadically found, but a possible relationship was suggested with a past history of periodontal disease (Mombelli & Mericske-Stern, 1990; Quirynen & Listgarten, 1990; Sordyl et al., 1995; Lee et al., 1999), but only if teeth remained. Nakou et al. (1987) and Sbordone et al. (1999) assessed the flora in patients with a previous history of periodontal disease but who had been completely edentulous at the time of the implant placement. They were unable to detect key pathogens such as *P. gingivalis*, *A. actinomycetemcomitans* or gram-negative organisms generally.

In partially dentate individuals, however, a relationship appears to exist between pocket depth and plaque composition and this may include both teeth and implants. Thus, shallow periodontal or peri-implant pockets harbor predominantly gram-positive cocci (*Streptococcus* spp.) and rods suggesting health, while pockets deeper than 5 mm most frequently harbor pathogenic species including *Porphyromonas gingivalis*, *Prevotella intermedia* and *Fusobacterium nucleatum* and others (Becker et al., 1990a; Hickey et al., 1991; Palmisano et al., 1991; Mombelli et al., 1995; Papaioannou et al., 1995, 1996; Mombelli & Lang, 1998; Leonhardt et al., 1999; Listgarten et al., 1999).

Implants generally also have the problem that they have a subgingival “microgap” at the connection of implant root to prosthodontic abutment, and this gap can provide a comfortable nook for subgingival plaque maturation (Quirynen et al., 1993b, 1994b;
Jansen et al, 1997). Such gap-related microbial deposits have been suggested to lead to bone loss around implants (Lindquist et al, 1988).

The relationship between organisms causing periodontal disease and those associated with dental implant failure means that proper control of the former must be maintained to prevent the latter. Natural teeth (along with the tongue [Lee et al, 1999] and other oral mucosal surfaces) are a major reservoir for pathogens, and differences in flora around dental implants placed in the fully edentulous patient vs. the partial edentulous patient have been demonstrated. Streptococcus spp., Capnocytophaga, Veillonella spp., Peptostreptococcus spp. (Mombelli et al, 1988; Leonhardt et al, 1993; Augthun et al, 1997; Lee et al, 1999) and sporadically Fusobacterium spp. and Prevotella intermedia (Mombelli et al, 1987; Danser et al, 1997) are the major components of peri-implant bacterial deposits in completely edentulous patients. For dental implants placed in partially edentulous patients Porphyromonas gingivalis, Prevotella intermedia, Fusobacteria spp., and spirochetes (Simons et al, 1993; Quirynen et al, 1996; Govoussis et al, 1997; Kalykakis et al, 1998) have been reported.

In addition to periodontal disease, the possibility also exists that previous or current periapical pathology of the remaining teeth may also have an impact on success with dental implants. It has been demonstrated that previous peri-radicular pathology of the tooth being replaced by the implant or present peri-radicular pathology of the tooth adjacent to the implantation site can negatively influence outcomes (Sussman, 1997; Shaffer et al, 1998).

**Differences in geometry of collars and transgingival abutments vs. the endosseous segment meant to promote osseointegration.**

Dental implant systems are routinely fabricated from either commercially pure titanium (CpTi) or titanium alloy (Ti-6Al-4V) and generally consist of an implant root component (sometimes called a “fixture”) and a transgingival abutment component. The surface geometry of these components will differ depending upon the tissue expected to be in
contact with them. The implant root usually has a coronal collar region (one or more mm in length) that is meant to contact peri-implant soft tissue when the implant goes into function, while the remainder or at least majority of its length is meant to be submerged in bone and to achieve implant fixation to bone (i.e. “osseointegration”). The most common surface geometry for the soft-tissue interfacing zones of an implant system (including the collar region of the implant root and the transgingival abutment) is a machined, highly polished surface with a roughness average (Ra) or center line average (CLA) of 0.05-0.2 μm. The rationale here is that this surface finish will optimize soft tissue health by minimizing dental plaque attachment to these surfaces (Bollen et al, 1996). Alternatively, it has been proposed that these soft tissue interfacing surfaces should be prepared with circumferential horizontal grooves of a certain size range (10μm) to promote gingival fibroblast migration and orientation in relation to the implant surface and even to inhibit apical migration of epithelium as demonstrated by Chehroudi et al (1989) in implants placed percutaneously in rat calvaria.

The surface geometry of the bone-interfacing segment of an implant root generally will be prepared with features such as threads, undercuts or surface irregularities that allow mechanical interlocking with bone, which is the basis of osseointegration. The original dental implant device introduced by Branemark and colleagues (Branemark et al, 1977) used a threaded implant with a machined surface finish to achieve osseointegration. While this worked very well in situations where the recipient bone was fairly dense and of sufficient height to receive a long fixture (e.g. at least 13mm in the maxilla and at least 10mm in the mandible, van Steenberghe et al, 1990), a machined surface finish was not well suited for short implant lengths or in bone of low density. Others (e.g. Deporter et al, 1990, 1996; Pilliar et al, 1998a) have since shown that implants that allow secure mechanical interlock of bone with the implant as a result of the implant surface geometry are better for these more challenging clinical situations as they increase implant surface area available for frictional contact with bone and, at least for implants with a porous-surfaced geometry, allow for 3-dimensional bone ingrowth. Textured surfaces prepared by acid-etching, grit-blasting, plasma-spraying or sintering also are thought to promote better osteoconduction in early wound healing (Davies 1998; Simmons et al, 1999) and
more secure long-term osseointegration (Deporter et al, 1996; Cochran, 1999). All of this, however, relies on the textured surfaces being initially fully submerged in bone and remaining so throughout the functional life of the implant. Should this not be the case, any rough implant surface or porous region that becomes exposed to the oral environment is likely to become colonized by dental plaque resulting in a peri-implant infection that may progress to implant failure as is reviewed below.

Surface texture can be created either by removing or applying material to a machined surface (Pilliar et al, 1998b). Acid-etching or grit-blasting are examples of ways to create textures by removing surface material, while plasma-spraying (with titanium, titanium alloy or calcium phosphate) and sintering are examples of adding material to create surface textures or geometries. The texture or geometry created again depends on the technique used and ranges from 1-2μm pits with acid-etching or irregularities of 20 to 50 μm for plasma-spraying to 100μm or more for sintering. Sintering has the added advantage of providing a significant surface porosity into which bone can grow in a 3-dimensional fashion providing for a much more extensive mechanical interlocking ("osseointegration"). Table 1 summarizes these various surface characteristics used for currently available dental implant systems (after Pilliar, 1998b)
Table 1- Surface topography of the current used dental implants

<table>
<thead>
<tr>
<th>Surface design</th>
<th>Appearance of surface features</th>
<th>Dimensions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Machined Ti or Ti alloy</td>
<td>Macropscopic- threads, vents, serrations</td>
<td>-mm range</td>
</tr>
<tr>
<td></td>
<td>Microscopic- parallel machining lines, pits, gouges, protrusions</td>
<td>-0.1 μm width/depth</td>
</tr>
<tr>
<td>Shot-blasted Ti or Ti alloy</td>
<td>Macropscopic- threaded or non-threaded</td>
<td>-mm range</td>
</tr>
<tr>
<td></td>
<td>Microscopic- heavily plastic deformed</td>
<td>-10 μm or less</td>
</tr>
<tr>
<td>Acid-etched Ti or Ti alloy</td>
<td>Macroposcopic- threaded or non-threaded</td>
<td>-mm range</td>
</tr>
<tr>
<td></td>
<td>Microscopic- etch-pits, dimple-like depression</td>
<td>-1 μm or less</td>
</tr>
<tr>
<td>Plasma sprayed Ti</td>
<td>Macroposcopic- threaded or non-threaded</td>
<td>-mm range</td>
</tr>
<tr>
<td></td>
<td>Microscopic- depressions, pores (isolated), undercut regions,</td>
<td>-20 μm or less</td>
</tr>
<tr>
<td></td>
<td>protrusions, sharp asperities</td>
<td></td>
</tr>
<tr>
<td>Plasma sprayed HA</td>
<td>Macroposcopic- cylindrical for press-fit</td>
<td>-50 μm or less</td>
</tr>
<tr>
<td></td>
<td>Microscopic- irregular depressions, protrusions, asperities, isolated pores</td>
<td></td>
</tr>
<tr>
<td>Porous-surfaced Ti alloy</td>
<td>Macroposcopic- tapered cylindrical for press-fit</td>
<td>~100 μm pore openings</td>
</tr>
<tr>
<td></td>
<td>Microscopic- interconnected pores, surface striations (thermal</td>
<td>-1 μm or less</td>
</tr>
<tr>
<td></td>
<td>etching), sinter neck zones</td>
<td>-40 μm diameter</td>
</tr>
</tbody>
</table>

Effects of implant surface geometry on dental plaque accumulation

Evidence exists to support the conclusion that surface topography or geometry and perhaps surface chemistry of dental implant components will affect susceptibility to dental plaque attachment and accumulation. For example, Quirynen et al (1993a) observed by differential phase-contrast analysis and DNA probe analysis in a study on patients that rough abutments (Ra= 0.81 μm as determined by a Perfhometer) supra- and subgingivally were loaded with 25 times more bacterial cells than smooth ones (Ra= 0.35 μm) over a three-month period in which regular oral hygiene was performed. The microbiota harbored on the rough surfaces were characterized by reduced numbers of coccoids indicating mature plaque. The same author (Quirynen et al, 1996) compared different degrees of roughness of titanium abutments and concluded that the threshold of
surface roughness above which significant bacterial adhesion and colonization could be expected was $R_{a} = 0.2$ microns, the usual upper limit of roughness for machined surfaces.

Gatewood et al (1993) studied the sequence of adherence of the microorganisms of the supragingival plaque on smooth titanium abutments when compared to tooth. The specimens ($6.5 \times 2 \times 1$ mm) were obtained from titanium implants with smooth transmucosal collars and plasma-sprayed endosseous posts of titanium or hydroxyapatite. Unerupted third molars were sectioned to similar dimensions. Ten patients with post-treatment pocket depths of $> 6$ mm on three non-adjacent teeth were selected and each had one set of the three specimen types bonded by random assignment to the selected teeth. Specimens were positioned so that the smooth titanium surfaces were supragingival and titanium or hydroxyapatite plasma-sprayed surfaces were subgingival. The patients were asked to discontinue all oral hygiene procedures and the test abutments were removed, two at a time, after 1, 3, 5, 7 and 10 days. Gatewood et al (1993) reported a similar sequence of adherence of the microorganisms of the supragingival plaque on smooth titanium abutments when compared to tooth enamel as described by SEM (the surface roughness for smooth titanium and enamel was not measured). They also reported that subgingival rough implant surfaces, i.e. titanium plasma-sprayed or HA plasma-sprayed, with roughnesses varying from 5 to 200 $\mu$m, presented the same sequence of adherence and maturation of the plaque as those occurring on cementum which is described as having a relatively flat contour, with a granular surface appearance. A few localized surface concavities of 100 to 300 $\mu$m were observed, probably representing areas of root resorption. However, the microbial species observed in relation to the two different surfaces, smooth and rough, indicated more mature plaque on the latter as assessed by SEM, supporting the findings of Quirynen et al (1993a).

Andersson et al (1999) presented results of a prospective multicenter study involving 50 standard machined Ti and 53 ceramic abutments (CerAdapt, Nobel Biocare) placed on Branemark implants and used to support 36 fixed partial prostheses (19 on ceramic and 17 on titanium abutments). After a 2-year follow-up, the authors found no differences
between ceramic and titanium abutments regarding bleeding of the peri-implant mucosa even though more plaque (rated “1”= none to “3”= visible directly) was recorded around Ti than ceramic abutments. There was minimal mean marginal bone loss recorded for both abutment types after 1 year, slightly greater with titanium (0.4 mm) than with ceramic (0.2 mm) abutments. Given the large standard deviations inherent in this kind of data, however, the differences were not statistically significant (p>0.05). In terms of quantity of plaque recorded around titanium and ceramic abutments, the study of Andersson et al is not in agreement with Rasperini et al (1998) who assessed colonization on titanium and ceramic abutments using SEM and failed to detect any differences between the two. These different conclusions by the two investigators might be explained by the fact that the assessment method used by Andersson et al (1999) was less precise than the SEM assessment used by Rasperini and colleagues.

It is also known that abutment surface modifications will occur during routine professional cleaning and that different techniques and instruments will affect implant surfaces differently (Mengel et al, 1998). Rapley et al (1990) used ten Branemark titanium abutment cylinders with one serving as an untreated control and the remainder being treated with a number of cleaning methods. He reported that rubber cup with flour of pumice created a smoother surface than the control abutment while an interdental tapered brush (Oral-B, Redwood City, CA), a soft nylon toothbrush (Dental H® Dental Hygiene, Santa Clara, CA), a plastic scaler (DIA 238, Nobelpharma, Chicago, IL), an Eva® plastic tip (Uniteck, Monrovia, CA), or a Cavi-jet® (Dentsply, York, PA) left a surface comparable to that of the control. By contrast, metal periodontal scalers and an ultrasonic scaling device (Cavitron® Dentsply, York, PA) created more severely modified surfaces indicating that their use should be avoided.

Given that plaque accumulation is related to the degree of surface roughness (Quirynen et al, 1993a), one could anticipate that porous implant surfaces would certainly promote plaque accumulation if they were to become denuded of bone and exposed to the oral environment. This is supported by a study reported by Deporter and coworkers (1988) where porous-surfaced implants were placed in dog mandibles using a two-stage surgical
approach. In this study, the implant root component was fully porous-coated (i.e. had no machined coronal collar region), and this was fully submerged in bone at the time of implant placement. As well, the apical one-third of the transgingival abutment (connected to the implant root at the second or re-entry surgery) was prepared with a porous surface (the coronal two-thirds being machined) with the intention that this short porous segment on the collar might permit gingival fibrous connective tissue ingrowth and the formation of a true peri-implant gingival attachment. In a minority of the retrieved specimens this desired outcome occurred; however, in the majority of sites the porosity of the collar simply became contaminated with dental plaque at the time of re-entry surgery and abutment connection leading to peri-implantitis and failing implants.

One can conclude then that the surface roughness of the collar segment of a dental implant root and of transgingival abutments both play a role in potential plaque accumulation thereby influence the future success or failure of an implant. Implant surfaces that have the capacity to harbor plaque and that cannot be cleaned effectively with routine daily hygiene practices should not be exposed to the oral environment. In addition, the possibility of localized crestal bone loss due to mechanical effects (overloading or stress-shielding) would make initially buried implants zones susceptible.

The key to success with dental implants that utilize textured or rough surfaces to improve osseointegration, then, is to design them so that the rough surfaces always remain in bone. For example, one manufacturer (3I Corporation, West Palm Beach, Fl) uses an acid-etched surface finish to improve osseointegration on its threaded dental implants (Cochran et al, 1996; Lazzara et al. 1999). However, the coronal-most threads are not acid-etched so that if as anticipated one or two threads become denuded of bone following initial healing and crestal bone remodeling, the affected exposed threads do not have a surface likely to promote serious plaque accumulation. Supporting this approach are the results of a retrospective study by Wheeler (1996) in which he followed the progress of cylindrical-shaped, press-fit implants utilizing a plasma-sprayed Ti or HA surface to achieve osseointegration. All of these implants had a machined collar length of only 0.5-0.75 mm and, despite early success, over the eight-year observation period a fair
number of these implants developed problems. Crestal bone remodeling, possibly related to the establishment of "biologic width" (Abrahamson et al, 1996; Berglundh et al, 1996; Cochran et al, 1997), led to denudation of bone from the plasma-sprayed surfaces with attendant plaque infection, progressive bone loss and implant failure. There were differences observed between Ti and HA plasma-sprayed surfaces. Thus, the titanium plasma-sprayed implants failed more frequently in the first two years of implant function while the HA-plasma-sprayed implants took longer to fail. However, by 8 years far more HA-coated implants had failed in this study. Why this should be has not been determined, but may relate to greater porosity inherent in the HA coatings (Wennberberg et al, 1993) or even to delamination of the HA layer as observed by electron microscopy assessment by David (1995). Jovanovic et al (1993) reported resorption of the HA-coating from Integral®, implants (Calcitek, Carlsbad, CA) by inflammatory phagocytosis. Others have reported data supporting Wheeler’s observations (Jones et al, 1997; Watson et al, 1999).

Several investigators have studied the effect of surface roughness on the progression of experimental ligature-induced peri-implantitis in animals. For example, Cook & Rust-Dawicki (1995b) used this model in dogs (silk ligatures) over a 26-week period to compare the performance of what the author called "cancellous-structured titanium-coated" (CSTi) 10 mm long cylindrical dental implants (0.5 mm coronal smooth ring, 2 mm apical grit-blasted and the remaining 7.5 mm with so called cancellous titanium coating) and hydroxyapatite-coated implants (Calcitek, Integral design without holes was used as control). Results indicated no significant differences in terms of crestal bone loss as assessed by direct comparison of radiographs after sacrifice to those taken at the base line. Both implant types and also both ligated and non-ligated specimens showed crestal bone loss. When comparing the bone loss in ligated vs. non-ligated specimens within the CSTi group, linear measurements on histological sections from the top of the implant to the bottom of the defect done 26 weeks after the ligature placement revealed a mean crestal bone loss of 1.46 mm (SD= 1.62) for the specimens on the non-ligated group and 1.96 mm (SD= 0.94) for the ligated one. However, the differences calculated with paired t-test failed to reveal statistical significance (p= 0.3346) between the two groups. For the HA-coated group, a mean 1.26 mm (SD= 1.23) crestal bone loss was measured on non-
ligated specimens and 1.64 mm (SD=0.39) on the ligated one, 26 weeks after the ligatures placement. Again, paired t-test failed to demonstrate statistically significant differences between the two groups (p= 0.0986). Histological assessment, however, revealed that the HA-coated specimens showed delamination or breakdown of the HA-coating and dissipation of HA particles into the surrounding tissues.

Tillmanns et al (1998) compared the susceptibility of machined titanium vs. titanium plasma-sprayed or HA-coated implants in a similar model in dogs, and in support of Cook concluded that all three types were equally susceptible to plaque contamination and bone loss in experimental peri-implantitis. However, it is generally agreed that this model is somewhat different than naturally occurring peri-implantitis in humans since it is primarily the physical irritation of the applied ligatures that is responsible for progression of “disease”. It is most likely that once the ligatures have been removed, machined surfaces would suffer less spontaneous progressive bone loss than either the Ti- or HA-plasma-sprayed ones, but this was not studied. Lindhe and Ericsson (1978) reported that bone levels in the dog mandible stabilized around teeth when ligatures were removed, while Marinello et al (1995) in a ligature-induced peri-implantitis model reported continuing bone destruction for 3 months after ligature removal, resulting in implant loss in some instances.

Callan et al (1997) reported an average follow-up period of 4.2-year of 203 patients who received 350 implants (173 HA plasma-sprayed and 177 Ti or Ti alloy threaded machined) on which prostheses were constructed. They concluded that implant surface composition does not seem to have too much effect on circumferential bone loss (>3mm were recorded for 76.9% of the implants in the HA plasma-sprayed group and 77.4% in the Ti or Ti alloy group). This study is not in agreement with Wheeler’s findings possibly because the follow-up period was shorter in this case. Also, HA-coated and titanium implants group populations were not equal although the difference was not great. In this study 177 titanium implants were used as compared with only 173 HA-coated implants. For the threaded implants, like the HA-plasma spray-coated ones, if the screw threads were exposed to the sulcus or perio-pocket environment, failure was more likely to occur.
From this it may be concluded that once any surface becomes denuded of bone it will lead to undesirable bone loss and unacceptably high implant failure rates according to established and accepted criteria, i.e., greater than 1.5 mm in the first year and more than 0.2 mm for every subsequent year (Albrektsson et al, 1986; Smith & Zarb 1989).

Callan et al (1998) also reported that the level of the implant-transmucosal abutment interface ("microgap") when placed subgingivally resulted in an increased crestal bone loss, with no correlation with the implant surface characteristics.

**Biomaterial centered infection**

Bacterial adherence and colonization have been considered key factors in the pathogenesis of biomaterial-based infections. As previously discussed, bacterial attachment to implant surfaces depends on the type of bacteria, the physical or chemical characteristics of the implant surfaces, the presence or absence of oral fluids interposed between the bacteria and the implant surface.

Implant infection, also called "foreign body effect" has the following features (Gristina & Naylor, 1996):

- Biomaterial/damaged tissue substratum
- Adhesive bacterial colonization to the substratum
- Resistance to host defense mechanisms and antibiotic therapy
- Characteristic bacteria like *S.epidermidis* or *Pseudomonas aeruginosa*
- Specificity of phenomena (material, organism, host location)
- The transformation of opportunistic pathogens into virulent microorganism by the presence of biomaterial substratum
- Polymicrobaility
- Persistence of infection until removal of the substratum
- Absence of tissue integration at the biomaterial-tissue interface
- Presence of tissue cell damage or necrosis
Implant centered infections are polymicrobial (Gristina et al., 1985) and the most frequent encountered species are: *S. aureus*, *S. epidermidis* and *Pseudomonas, Enterococcus, Streptococcus, Bacillus* and *Proteus* species. Microorganisms are exposed to the surface of the biomaterial or of a foreign body by direct contamination, contiguous spreading or hematogenous seeding. The initial attachment is a non-specific, reversible interaction. It depends on chemical and physical characteristics of bacteria, biomaterial and the interposing fluids. The biomaterial presents high-energy sites or rough areas that represent preferred sites for bacterial adherence. Usually, the biomaterials and the bacterial cells are negatively charged repelling each other. Other attractive forces, i.e., van der Waals and hydrophobic, facilitate the positioning of diverse cells, including bacterial cells, closer to the biomaterial surface. Proximity of the bacteria and the biomaterial surface will further facilitate the action of chemical bonding, i.e., ionic, hydrogen and covalent. Subsequent to attachment, specific receptor-mediated bacteria-substrate interactions may occur, based on chemical and hydrophobic bonds (Christensen et al., 1985). Also, some surface characteristics of bacteria, i.e., the presence of pili and fimbria, will help their adherence.

Microorganisms in colonies on surfaces form layers, two to hundreds of organisms thick composed of cellular material, extracellular polysaccharide, environmental absorbates and debris. In the most common situation, bacteria form a three-dimensional array and bond together by the existence of extracellular matrix proteins. The implanted biomaterials are rapidly coated by constituents from serum such as fibronectin, osteonectin, vitronectin, albumin, fibrinogen, laminin and collagen, the same components of the intercellular matrix. All these suggest that the moiety for bacterial adherence is present from the moment of material implantation, the start of the infection being just a matter of bacterial contamination and then the development of an imbalance between the microorganisms and defense capacity of the host.
3) Ligature-induced peri-implantitis

Many experiments have been reported that rely on animal models to study diverse periodontal and peri-implant pathology. There is general agreement that the dog is an excellent experimental animal in which to study gingival and periodontal disease and also peri-implantitis, dogs being small and easy to handle animals. Their oral tissues, especially the gingival-dental junction and their periodontium, are quite similar to those of man, thus making comparison possible in different situations. Dogs are susceptible to development of gingivitis and periodontal disease like humans, the cause of these being microbial. There are experimental studies in the literature that confirm the microbial implication in periodontal tissue breakdown in dogs. The microorganisms promote the ulceration of the epithelium lining the gingival sulcus and this will shortly lead to bone loss. The microbial species found in perio-compromised dogs are similar with those responsible for periodontal tissue degradation in humans, i.e. P. gingivalis, and notable difference exists in flora associated with healthy and diseased areas (Page & Schroeder, 1982).

The usual way to promote experimental peri-implantitis in an animal model is by means of cotton or silk ligatures tied around the implant abutment and meant to promote increased levels of subgingival plaque organisms and crestal bone loss around the affected implants. A number of authors have done this with implants of differing surface geometry and then used the diseased sites to test various "rescue" procedures i.e., surgical debridement, GTR or GBR, procedures meant to encourage restitution of lost osseointegration. This approach is discussed more extensively below as the method was used in the present study.

Rovin and his colleagues (1966) appear to have been the first to describe a model for inducing periodontal destruction by ligature placement around teeth. They described progressive periodontal breakdown in rats caused by the combined presence of a size 000 braided silk ligature and the microorganisms that became attached to these ligatures. Their model was subsequently adopted for use in other animals. For example, Ericsson
(1975) described a method for inducing rapidly progressive, reproducible periodontal lesions around teeth in dogs. A ligature of cotton floss was placed around the teeth at the cemento-enamel junction, and the dogs placed on a soft diet conducive to gross plaque accumulation. Radiographs and sections prepared 32 weeks later revealed that extensive breakdown of the periodontal tissues had occurred. Subsequently, Lindhe & Ericsson (1978) reported that removal of the ligatures after one month of disease induction converted the "active, progressive periodontitis...into a resting, non-progressing lesion", demonstrating that indeed the ligatures were the main promoter of inflammation and tissue destruction.

Brandes and co-workers (1988) appear to have been the first to use the described model to induce peri-implantitis (ITI hollow cylinder implants). In their study it was demonstrated radiographically that 20-50% of the peri-implant crestal bone can be destroyed within 6 to 20 weeks following ligature placement assuming a soft diet and cessation of hygiene procedures are also part of the study design (Lindhe et al, 1992; Grunder et al, 1993; Schupbach et al, 1994; Marinello et al, 1995; Ericsson et al, 1996a; Persson et al, 1999). All the above quoted authors used cotton ligatures, although the results in terms of bone loss did not seem to be influenced by the type of ligature used, i.e. cotton or silk (Jovanovich et al, 1993; Hurzeler et al, 1995; Wetzel et al, 1999). The bone loss was generally horizontal, with more of a saucer shape around the implants (Grunder et al, 1993). Some authors reported vertical, circumferential-type defects (Jovanovic et al, 1993; Hurzeler et al, 1995), while others suggested that the pattern of bone resorption seems to be influenced by the distance between the implants, with the recommended distance being 3.5 mm for vertical defect formation (Adell et al, 1985). Grunder et al (1993) used the recommended distance between fixtures in a study of ligature induced peri-implantitis and that resulted in horizontal bone loss, possibly due to overlapping that occurred between defects from neighboring fixtures.

A study by Marinello et al (1995) summarizes the clinical and histological events that occur during ligature-induced peri-implantitis around Branemark implants in dogs. Within 4 to 6 weeks after cotton ligature placement, an active destructive inflammatory
lesion was present in the soft and mineralized tissues around the implants with approximately 25% of crestal bone height having been lost. By 10 to 12 weeks bone loss had continued to the point where implants became unstable and were lost. For the implants that were not lost by this time, histological assessment revealed an active lesion with much osteoclastic activity on the surface of the bone crest.

4) Proposed “rescue” procedures to manage experimentally-induced periimplantitis

A number of studies have been undertaken to investigate possible “rescue” procedures for dental implants following the establishment of ligature-induced peri-implantitis. Two objectives must be met with these procedures. Firstly, the exposed and affected implant surfaces need to be decontaminated as they are generally colonized by pathogenic, primarily gram-negative microorganisms and secondly, lost supporting bone needs to be regenerated if possible to re-submerge the decontaminated implant surfaces.

Methods for implant surface decontamination

Implant surface decontamination has been addressed using physical methods and/or topical agents applied directly to the affected surfaces and/or by the use of systemic antibiotic administration. Physical methods tested have included the CO₂ laser (Kato et al., 1998) and the air-flow powder instrument (Zablotsky et al., 1992a), while topical chemotherapeutic modalities tested have included citric acid, chlorhexidine gluconate, hydrogen peroxide, tetracycline HCl, stannuous fluoride, chloramine-T, polymixin B and delmopinol (Zablotsky et al., 1992a).

Zablotsky et al (1992a) analyzed in vitro the facility of several approaches to detoxify surfaces of grit-blasted titanium alloy and HA-coated test strips that had been contaminated with radioactive ¹⁴C- labeled E.coli endotoxin (lipopolysaccharide, LPS). The authors reported that the most effective method in terms of reducing the number of LPS counts/min/sq.mm was the air-powder abrasive instrument for the metallic surfaces
and citric acid for the HA-coated strips. These findings might not be too conclusive, however, because as indicated by the investigators, less LPS adhered initially to the metal than to the HA-coated surfaces. The same author (Zablotsky et al, 1992b) studied surface modifications of plasma-sprayed HA-coated implants following treatment with citric acid, chlorhexidine gluconate, hydrogen peroxide, tetracycline HCl, stannuous fluoride, chloramine T, polymixin B or a prototype plastic cavition tip. The specimens were macroscopically, microscopically (SEM) and spectrometrically assessed. HA substrate bond strength and dissolution testing were also performed for the surfaces treated with supersaturated citric acid solution. Summarizing the results, it seems that all the treatments reduced surface roughness when viewed by SEM and that significant changes in Ca/P ratios occurred with the HA-coated implants, although no treatments altered their crystallinity. Up to 60 seconds of citric acid treatment left significantly greater HA-coating thickness than all other treatments and did not alter the tensile bond strength of coating-to-substrate interface.

Dennison (1994) compared in vitro decontamination of machined or plasma-sprayed titanium and HA-coated implants contaminated with P.gingivalis endotoxin (labeled with \textsuperscript{135}I) using water, citric acid, chlorhexidine or an air-powder abrasive. He reported that machined surfaces are more easily decontaminated than the two other surfaces by all of the treatments tested. Citric acid was equally effective in decontaminating machined titanium and HA-coated surfaces, while HA-coated implants could be disinfected with the same efficiency by air-powder abrasive and by citric acid.

A number of investigators have also done in vivo studies in animal models and reported the successful use of some decontamination procedures in managing peri-implantitis, namely, an air-flow powder instrument (Grunder et al, 1993; Schupbach et al, 1994; Hurzeler et al, 1995; Machado et al, 1999), air-powder abrasive instrument and followed by citric acid (Jovanovic et al, 1993), Chlorhexidine 0.12% (Wetzel et al, 1999) and delmopinol (Persson et al, 1996). In humans, chlorhexidine 0.12% (Lehmann et al, 1992; von Arx et al, 1997) and tetracycline solution 50mg/ml (Mellonig et al, 1995) have been used with success.
Investigators have also used systemic antibiotic administration in animal models of peri-implantitis in an attempt to rescue implants affected by this condition. For example, Persson et al (1999) and Ericsson et al (1996a) used Amoxicillin and Metronidazole administered for 3 weeks, and after that, local debridement, to “rescue” Branemark-type implants placed in dogs and affected by ligature-induced peri-implantitis. The results were histologically assessed and consisted of healing of the peri-implantitis lesion, marginal recession of the peri-implant mucosa and in some cases, new bone formation. The results suggested that systemic administered antibiotics reduce the number of periopathogens and help restoring the health of the peri-implant tissues.

Mombelli and Lang (1992) used systemically administrated Omidazole (1000mg/day for 10 days) combined with surgical debridement with definitive decontamination using 0.5% chlorhexidine in patients with naturally-occurring peri-implant lesions that had initially demonstrated cultivable Prevotella intermedia. The treatment outcome was an immediate reduction of bleeding and inflammation and the sites remained negative when tested for P. intermedia 1 year later. Antibiotic administration has also been reported by other investigators (Lehmann et al, 1992; Hammerle et al, 1995; von Arx et al, 1997) as adjuvant therapy for debridement or regenerative techniques around implants, the most frequently used drugs being Amoxicillin, Metronidazole or Omidazole.

**Techniques to regenerate bone around peri-implantitis-affected dental implants**

Much has been written on regenerating bone around dental implants. The majority of investigators have followed the principles of Guided Bone Regeneration (GBR) first described by Hurley et al (1959) in the orthopedic literature and later by others in relation to dental implant-related, ridge augmentation procedures and treatment of peri-implantitis (Buser et al, 1990a; Nyman et al, 1990; Mellonig & Triplett, 1993; Vlassis et al, 1993). The rationale behind GBR is the exclusion of gingival epithelium and soft connective tissue by the use of a physical barrier while allowing for the migration and differentiation of osteoprogenitor cells from the surrounding and barrier-isolated alveolar bone. The most commonly used barrier materials for this approach have been non-resorbable
millipore filters and Gore-Tex augmentation material (GTAM), which are forms of e-PTFE (expanded polytetrafluorethylene) (W.L. Gore and Sons, Flagstaff, AR), but other materials used for this purpose include bioresorbable barriers such as Vicryl mesh, demineralized freeze-dried lamellar cortical bone strips, freeze-dried dura mater (Zablotsky et al, 1992 c) and cross-linked bovine collagen (Meffert, 1992).

GBR techniques have been used successfully at the time of implant placement to augment inadequate bone width (Andersson et al, 1993), to manage peri-implant gaps following immediate implant placement into tooth extraction sites (Becker & Becker, 1990b), to treat fenestrations or dehiscences around threaded and HA-coated dental implants (Stentz et al, 1997) and to "rescue" threaded and rough-surfaced implants, i.e., titanium plasma-sprayed, sand-blasted and acid-etched implants (Lehmann et al, 1992; Grunder et al, 1993; Hammerle et al, 1995; Wetzel et al, 1999). Barrier materials have either been used alone or in conjunction with one or more graft materials with osteoinductive and/or osteoconductive properties. Four major groups of graft materials are available for clinical use and include autografts, allografts, xenografts and alloplastic materials. An autograft is considered the ideal material for regenerating bone because it has osteogenic, osteoconductive and likely osteoinductive potential. Allografts, when properly prepared, are osteoinductive as well as osteoconductive. Xenograft materials have osteoconductive properties. Both allografts and xenografts are preferred in a demineralized state, knowing that the hydrochloric acid used for demineralization facilitates the activity of the bone morphogenetic proteins that encourage bone cell differentiation and bone formation. The alloplastic materials are natural or synthetic calcium-phosphate-based ceramics. They are osteoconductive only (Mauro F, 1999).

A number of investigators have studied GBR techniques in the management of peri-implantitis in animal models, and many of these are summarized in Table 2.
Table 2- Management of peri-implantitis; animal studies

<table>
<thead>
<tr>
<th>Author/Animal model</th>
<th>Implant surface type</th>
<th>Peri-implantitis model</th>
<th>Decontamination procedure</th>
<th>Antibiotic regimen</th>
<th>Treatment procedure</th>
<th>New bone fill (linear measurements, mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schupbach et al. 1994 (dogs)</td>
<td>Acid etched</td>
<td>Cotton ligatures, 30-50% bone loss</td>
<td>Air flow powder instrument</td>
<td>Not reported</td>
<td>Debridement/ GTR (ePTFE)</td>
<td>Not reported</td>
</tr>
<tr>
<td>Grunder et al. 1993 (dogs)</td>
<td>Acid etched</td>
<td>Cotton ligatures, 30-50% bone loss</td>
<td>Air flow powder instrument</td>
<td>Not reported</td>
<td>Debridement/ GTR (ePTFE)</td>
<td>-0.1 (SD=0.1) (3 months)</td>
</tr>
<tr>
<td>Hurzeler et al. 1995 (dogs)</td>
<td>CPTi</td>
<td>4-0 silk ligatures, 30-50% bone loss</td>
<td>Air-powder abrasive instrument</td>
<td>Not reported</td>
<td>Debridement (D)/ D+HA/ D+FDB/ D+ePTFE/ D+ePTFE+HA/ D+ePTFE+FDB</td>
<td>0.5 (SD=0.3) 1.8 (SD=0.3) 2.2 (SD=0.4) 3.6 (SD=0.4) 3.2 (SD=0.6) 3.8 (SD=0.8) (4 months)</td>
</tr>
<tr>
<td>Machado et al. 1999 (dogs)</td>
<td>CPTi</td>
<td>Cotton ligatures</td>
<td>Air-powder abrasive instrument</td>
<td>Metronidazole 250 mg/day 21 days</td>
<td>Debridement (D)/ D+ePTFE/ Bone graft (BG)/ D+ePTFE+BG</td>
<td>0.85(SD=0.4) 1.37(SD=0.8) 1.6(SD=0.6) 1.58(SD=1.1)</td>
</tr>
<tr>
<td>Wetzel et al. 1999 (dogs)</td>
<td>Ti PS TiSBE MTi</td>
<td>4-0 silk ligatures, 40% bone loss</td>
<td>Chlorhexidine 0.12%</td>
<td>Metronidazole 20 mg/kg/day 10 days</td>
<td>Debridement (D)/ D+ePTFE</td>
<td>2.6(SD=0.6) 2.3(SD=0.8) 2.2(SD=1.1)</td>
</tr>
<tr>
<td>Jovanovic et al. 1993 (dogs)</td>
<td>CPTi Ti PS HA-coated</td>
<td>4-0 silk ligatures</td>
<td>Air-powder abrasive instrument and citric acid</td>
<td>Not reported</td>
<td>Debridement (D)/ D+ePTFE</td>
<td>Not reported</td>
</tr>
<tr>
<td>Persson et al. 1996 (dogs)</td>
<td>CPTi</td>
<td>Cotton ligatures 20% bone loss</td>
<td>Delmopinol</td>
<td>Amoxicillin 375x2 mg/day Metronidazole 250x3 mg/day 21 days</td>
<td>No treatment/ D+ePTFE</td>
<td>-0.32mm (4 months)</td>
</tr>
<tr>
<td>Singh et al. 1993 (micropig)</td>
<td>Not reported</td>
<td>Silk ligatures</td>
<td>Not reported</td>
<td>Not reported</td>
<td>e-PTFE D No treatment</td>
<td>2.13mm 1.37mm 0.87mm</td>
</tr>
</tbody>
</table>

Ti PS: titanium plasma sprayed; ePTFE: expanded PTFE membrane; TiSBAE: titanium sand-blasted acid etched; MTi: machined titanium. CPTi: commercially pure titanium
From this, it might be concluded that the grafting techniques are the best in terms of the re-gained bone height, followed by the GTR procedures using only membrane and no graft material. The worst performance was attributed to the debridement when this was used alone (Hurzeler et al., 1995; Machado et al., 1999). When the unresorbable membrane (e-PTFE) was used alone and was compared to the debridement alone, again, the latter gave poorer performance (Singh et al., 1993; Persson et al., 1996; Wetzel et al., 1999).

Some of the above listed authors reported results of light microscopy assessment. Schupbach et al. (1994) and Grunder et al. (1993) described similar outcome for the groups treated with debridement only and GTR. A gingival sulcus was formed; that extended apically to various depths. Connective tissue was interposed between the alveolar bone crest and the gingival sulcus. The free gingival margin was covered by stratified, squamous keratinizing oral epithelium. Coronally, the sulcular epithelium was continuous with the oral epithelium and apically with the regenerated junctional epithelium. This description resembles the normal architecture of the soft tissues that surround the implants that were never infected. Both authors reported new bone formation in the initial bone defect or contact with implant's surface (i.e., re-osseointegration) and none to few inflammatory cells. Jovanovic et al. (1993) reported similar findings to that mentioned above and also, they report the presence of active phagocytosis of the HA layer by macrophages and giant cells on larger areas of HA surfaced dental implants.

Persson et al. (1996) in a split-mouth design study used light microscopy to compare dog mandibular sides treated with GTR and ones in which no treatment was performed. These investigators revealed that the sides on which surgical technique was used presented the normal tissue architecture described above, as opposed to sites that did not receive any treatment, which were characterized by large inflammatory lesions, ulcerated pocket epithelium and inflammatory cells infiltrate: plasma cells, lymphocytes, macrophages, neutrophils, bacteria. Although new bone formation occurred in previous bone defects, the amount of re-osseointegration observed was small and at all experimental implant
sites, a thin connective tissue capsule was found to separate the implant surface from the newly formed bone.

These results from animal studies results confirm with those found in human case reports, as summarized in:

**Table 3- Management of peri-implantitis; clinical trials and case reports**

<table>
<thead>
<tr>
<th>Author</th>
<th>Surface type</th>
<th>Initial defect (linear measurement mm)</th>
<th>Decontamination procedure</th>
<th>Antibiotic regimen</th>
<th>Treatment procedure</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lehmann et al, 1992</td>
<td>TiPS</td>
<td>Probing depth 9 mm angular bone loss</td>
<td>Chlorhexidine 0.12% and saline solution</td>
<td>Amoxicillin 1.5g/day Ornidazole 1g/day</td>
<td>Debridement + e-PTFE</td>
<td>Gain 4-5 mm</td>
</tr>
<tr>
<td>von Arx et al, 1997</td>
<td>TiPS</td>
<td>5 mm vertical, bone loss</td>
<td>Chlorhexidine 0.5%</td>
<td>Amoxicillin 625x3 mg/day, 7 days Metronidazole 1.5g/day, 7days</td>
<td>Autogenous graft+ resorbable barrier (GUIDOR)</td>
<td>Defect filled with bone 6 month later (radio graphs)</td>
</tr>
<tr>
<td>Hammerle et al, 1995</td>
<td>TiPS</td>
<td>2.6-7.1 mm vertical bone loss</td>
<td>Chlorhexidine 0.12%</td>
<td>Amoxicillin 325x3mg/day, 10 days Metronidazole 250x3mg/day, 10 days</td>
<td>Debridement + e-PTFE</td>
<td>Mean bone gain 2.3 mm</td>
</tr>
<tr>
<td>Mellonig et al, 1995</td>
<td>TiPS</td>
<td>9 mm (probing depth 10mm)</td>
<td>Tetracycline solution 50mg/ml</td>
<td>Not reported</td>
<td>HA+ e-PTFE</td>
<td>Probing depth 1-2 mm</td>
</tr>
<tr>
<td>Mellonig et al, 1995</td>
<td>TiPS</td>
<td>10 mm</td>
<td>Tetracycline solution 50mg/ml</td>
<td>Not reported</td>
<td>DFDBA+ e-PTFE</td>
<td>Probing depth 3-5mm</td>
</tr>
</tbody>
</table>

**Ti PS:** titanium plasma sprayed  
**ePTFE:** expanded PTFE membrane  
**DFDB:** demineralized freeze-dried bone allograft  
All results are reported at the time of the membrane removal if not otherwise specified
As noted from Table 3, a number of authors reported varying degrees of success in the treatment in humans (Lehmann et al, 1992; Hammerle et al, 1995; von Arx et al, 1997) of peri-implantitis using either a resorbable (Biofix, polylactide) or non resorbable (Gore-Tex) barrier and a filling material which was either autogenous bone or xenograft. Reduction of probing depth was reported in all cases and also bone defects were filled with new bone as assessed radiographically. As in the animal studies, the clinicians used Chlorhexidine 0.12% or Tetracycline solution 50mg/ml for decontamination of the implant surface during surgery. Also, antibiotics were recommended (Amoxicillin & an imidazolic compound i.e., Omidazole or Metronidazole).

5) Results of previous studies in animals and humans using porous-surfaced dental implants

In the present study we have used a dental implant design with a novel surface geometry that provides superior resistance to tensile and other forces acting at the implant-bone interface (Vaillancourt et al, 1995). The implant root component has a tapered, truncated cone shape (taper angle 5°), a machined soft tissue-interfacing coronal collar segment and a porous-surfaced bone-interfacing region. Typically, the porous surface region (300 μm thick) consists of 2 to 3 particle layers bonded to each other and to the machined solid implant substrate. The result is a strong integral structure with a 3-dimensional interconnected surface porosity (30-40% volume porosity) and a pore size in the range 50-200 μm, conditions known to encourage rapid bone ingrowth (Bobyn et al, 1980).

Early animal studies (Deporter et al, 1986a&b; 1988) demonstrated, using radiographic and histological techniques, that such porous-surfaced dental implants become fixed to bone (i.e. osseointegrated) in an interval as short as 4 weeks after implantation. These studies also showed that the porous surface should never be exposed to the oral environment because the same topography that allows for secure fixation to bone by bone ingrowth also strongly encourages plaque accumulation if the porous-surfaced region is not buried in bone, a fact that will unconditionally lead to failure of these fixtures assuming that no rescue procedures are used.
Simmons et al (1999) compared the initial stages of healing (days 0 to 16) around plasma-sprayed vs. porous-surfaced dental implants placed in rabbit femoral condyle. His investigation concluded that at days-4 and -8 post-implantation, the early healing tissues (composed of fibrin and collagenous matrix) were more securely attached to the porous-surfaced implants having extended into the three-dimensional structure formed by the porous surface. As a consequence, the early attachment strength to porous-surfaced implants, as assessed by mechanical pullout tests, also was greater than with plasma-sprayed implants. As well, initial matrix mineralization leading to osseointegration occurred more rapidly with the porous-surfaced design. Thus, implants with a porous surface geometry appeared to offer superior performance during early healing around the bone-interfacing segment of the implant root.

Deporter et al (1990) reported on the subsequent loading and function of porous-surfaced implants using a histological comparison in the dog of porous-surfaced vs. machined threaded dental implants supporting fixed bridges in an 18-month functional period. These authors observed that the porous surface allowed for successful force transfer and minimal crestal bone loss which was restricted to the machined collar region of the implant, i.e bone had been lost to a point at or just coronal to the machined collar surface-to-porous surface junction. It was argued that this pattern of bone remodeling was most likely due to a “stress-shielding effect” associated with the machined collar region (Pilliar et al, 1991). This speculation was supported later by 2-dimensional finite element analyses (Vaillancourt et al, 1995) using available histological material (from studies by Deporter et al, 1986a, 1986b, 1988) and a computer model. The authors predicted that the machined coronal collar segment of the partially porous-surfaced implants would result in regions of stress-shielding. The lack of effective mechanical coupling with bone in this collar region of the implant was linked to observed pattern of bone loss and related to “disuse atrophy”. Once the remodeling bone crest approached the porous surface segment of the implant root, bone ingrown into the porous surface appeared to allow for effective stress transfer thereby minimizing further bone loss. These observations were limited by the use of a two-dimensional model to represent a three-dimensional system. However,
their interpretation of the data was later apparently supported by another experiment performed by Al-Sayyed and colleagues (1994).

Vaillancourt et al (1996) analyzed other possible factors that might induce crestal bone loss with partially porous-surfaced dental implants, and concluded that prosthesis design and the length of the machined segment were important. It was once again demonstrated that stresses in the crestal bone were low next to the machined aspect of the implants.

The study by Deporter et al (1990) also demonstrated clinical success for both threaded and porous-surfaced implants throughout the 18-month trial period. As well, a morphometric analysis of retrieved specimens revealed that for porous-surfaced implants, a smaller segment of the implant surface available for contact with bone was required for effective osseointegration, suggesting that shorter implant lengths could be used with this design (Pilliar et al. 1998).

A human clinical validation of the successful use of similar porous–surfaced dental implants was begun in 1989 and reported by Deporter et al (1996). In this prospective study, a group of 52 fully edentulous patients were each treated with three porous-surfaced dental implants placed in the anterior mandible and a complete implant-retained overdenture. The mean implant length used was only 8.7mm and the 5-year success rate was 93.4%. Changes in crestal bone height measured on standardized radiographs were minimal and the initial report revealed the mean bone loss to be 0.43 mm in year one, 0.17 mm in year 2 and 0.13 mm in year 3, and the pattern of crestal bone remodeling was consistent with previously published dog data (Deporter et al, 1990). Periodontal and subclinical mobility indices indicated good peri-implant soft and hard tissue health (Levy et al, 1996, 1997) and the measurements and implant survival rates equaled or surpassed those reported by other investigators for titanium threaded, plasma-sprayed or HA-coated dental implants (Buser et al, 1990b; Quirynen et al, 1991).

It can be concluded that sintered porous-surfaced dental implants offer unique advantages over many other dental implant designs. These advantages include a tapered, press-fit
shape allowing for easy placement in normal or compromised bone sites, very short healing period (as short as 4 weeks in dog mandible) possibly due to attachment and orientation of fibrin and other tissue elements in early wound healing into the porous surface, reliable and secure osseointegration achieved by 3-dimensional bone ingrowth into the porous surface, stable crestal bone levels during function once the crest approaches the vicinity of the junction of the machined collar and porous-surfaced regions of the implant. Nevertheless, on occasion porous-surfaced implants, like all other implant designs, may suffer from atypical degrees of bone loss and when this happens it may lead to exposure of porous surface and the risk of peri-implantitis and implant failure. It was the purpose of the present study, therefore, to examine and compare two GBR techniques in rescuing porous-surfaced implants affected by experimentally-induced peri-implantitis.
II. Rationale

Porous-surfaced dental implants have a unique surface geometry formed by sintering of powder particles of titanium alloy to a solid implant core. The resulting implant has the highest surface area/implant length of any endosseous dental implant used today and the ability to osseointegrate by developing an interdigitated, 3-dimensional interlock at the bone-implant interface. These surface characteristics offer significant clinical advantages including superior resistance to tensile and torquing forces, allowing the use of shorter fixtures and superior performance in bone of poor quality (low density).

When properly installed in bone, a porous-surfaced implant will have its entire porous surface submerged within crestal bone thereby minimizing the likelihood of any of it becoming exposed to the oral environment. If, however, any of the porous surfaces of such of an implant does become exposed to bacterial plaque and displays signs of infection, an experimentally validated treatment procedure should be available for clinical use in “rescuing” the affected implants.

The purpose of this experiment was to induce peri-implantitis around healthy, osseointegrated porous-surfaced dental implants using the ligature model validated by other investigators with other dental implant systems, and to assess two surgical “rescue” approaches for the treatment of this condition.

III. Objectives

1) To establish a model in dog mandible of peri-implantitis around porous-surfaced dental implants using the ligature approach.

2) To compare, using BSEM quantitative methods, two approaches for the treatment of this experimentally induced condition.
IV. Materials and methods

Implant design and fabrication

Implants used in the investigation were provided by Innova Corporation (Toronto). They were fabricated from Ti-6Al-4V and consisted of an endosseous root component, a transgingival collar, a collar-retaining screw and an expanded healing abutment. The implant root had a tapered, truncated cone shape (taper angle 5°). A porous surface treatment described below covered all but the most coronal 1.0 mm or smooth collar region (SCR) of the implant root. The coronal uncoated part (SCR) and the healing abutment had a machined surface. The implants were 6 mm in length with a maximum coronal diameter of 4.1 mm. The transgingival abutment was 4.5 mm in height and had an inverse tapered shape with a maximum diameter of 6 mm that tapered down to 4 mm on the part that connected to the implant root (Figure 1).

The porous surface implants were prepared by methods described previously (Al-Sayyed et al., 1994) by a solid state sintering (1250° C for 1 h in high vacuum) process with which Ti-6Al-4V particles of a size range 45-150 μm in diameter were applied to the solid machined implant core. To achieve a continuous contour at the junction between the machined SCR and porous surface regions of the implant, the diameter of the implant core intended to be coated was machined with a 0.25 mm recess relative to the coronal machined part. Typically, the porous surface region (300 μm thick) consisted of 2 to 3 particle layers bonded to each other and to the solid implant core substrate by the sintering treatment. The result was a strong integral structure with a 3-dimensional interconnected porosity (30-40% volume porosity) with a pore size in the range 50-200 μm, conditions known to encourage bone ingrowth (Deporter et al., 1986, 1988, 1990).

All implant parts were cleaned and sterilized using routine methods (Deporter et al., 1986).
Figure 1. - Implant designs
Experimental Design

Details of the experiment are summarized in Table 4. Five male beagle dogs (Marshall Farms, North Rose, NY, USA) of 12.7 to 15.0 Kg body weight were used in this study. As a preparatory measure, all dogs had their second, third and fourth mandibular premolars and first molars extracted bilaterally. This procedure as well all other surgical interventions including ligatures placement and change, radiographic and microbiological sampling, were performed with the animals under general anesthesia. For pre-anesthesia Atropine (MTC Pharmaceuticals, Cambridge, ON) 0.02-0.05mg/kg and Acepromazine (Ayerst Laboratories, Montreal, QB) 0.1-0.5mg/kg, were administered subcutaneous. It was followed by induction by Pentothal (Abbott Laboratories Ltd., Montreal, QB). 25 mg/kg, intravenous to effect the endotracheal intubation for anesthesia, which was maintained by 1-1.5% halothane (Halocarbon Laboratories, River Edge, N.J., USA) in oxygen (BOC Gases, Etobicoke, ON). After a healing interval of 16 weeks, each animal received three implants on each side of the mandible placed into the created edentulous spaces. Implants were installed using a two-stage surgical approach as in earlier investigations (Al-Sayyed et al, 1994), and were positioned so that there was a minimum inter-implant distance of ~5 mm.

Due to some production difficulties by the manufacturer with fabrication of the transgingival abutments, re-entry surgery was delayed leaving the implants to heal for 8 weeks instead of the intended 4 weeks. At the time of re-entry and abutment connection, hygienic procedures were initiated and included brushing the teeth and implant abutments three times/week. They consisted of brushing the entire mouth with tap water and pumice using a regular toothbrush and brushing with water and an Interplaque System (Bausch and Lomb). Interdental brushes (Butler Gum®, JO Butler Company, Guelph, ON) and flexible pipe cleaners saturated in chlorhexidine 0.10% were employed especially for cleaning the areas between and near the transgingival abutments. Four weeks after abutment connection, the oral hygiene routine was stopped. At this time, peri-implantitis was initiated by tying ligatures around each implant in such a way as to force each ligature apically in an attempt to disrupt the integrity of the peri-implant soft tissue seal.
and to promote subgingival plaque accumulation. Also, at this time a soft diet was introduced consisting of Purina Lab Chow (Canine Diet 5006) and Canned Derby Beef (Derby Pet Foods, ON) all soaked in warm water.

During this phase of the experiment, as indicated, no hygienic procedures were performed. As well, microbiological and radiographic assessments were done every three weeks at which time the ligatures also were replaced. For the radiographic assessment, we used a GENDEX apparatus model 4519 105 0077 G1 (GENDEX CORP. Des Plains, Illinois, USA), a custom-made film holder (which was screwed on the middle implant after the removal of its transgingival abutment), and KODAK ultra-speed D films, size 2 (EASTMAN KODAK Co., Rochester, NY, USA). The crestal bone level was followed until the porous surface region was visible. In some cases bone resorbed until half of the porous surface was exposed. The amount of bone resorption was not the same for all the implants.

The described conditions being met, the ligatures were removed after 6 weeks in two dogs and after 8 weeks in the remaining three dogs. At the time of ligature removal oral hygiene procedures were re-instituted. The intended surgical "rescue" procedures were performed two weeks later in the first two dogs, as planned, and four weeks after ligature removal in the remaining three dogs because of scheduling problems. In each instance, for one week before surgery and two weeks after, the dogs received Amoxicillin, 250 mg, and Metronidazole, 500mg, orally twice daily, a regimen known to reduce periodontopathogens (Ericsson et al, 1996a).

Details of the rescue procedures used are given below. All rescued implants were allowed to heal for 12 weeks during which hygienic procedures including swabbing the peri-implant soft tissues three times a week with 0.10% Chlorhexidine were reinstated.
### Table 4 - Experimental Design

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<td></td>
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<td></td>
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<td>7</td>
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<td>histological assessment</td>
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Implant Surgical Procedures

Implantation procedure

As indicated, three implants were placed into the edentulous sites created on each side of the mandible. Procedures used in previous studies (Deporter et al, 1986, 1988, 1990; Al-Sayyed et al, 1994) were employed. This included making an incision in the buccal vestibule and elevating a lingually based full-thickness mucoperiosteal flap to expose the edentulous alveolar ridge. Sockets for all implants were created by drilling with a surgical handpiece and appropriate burs (round, pilot, implant burs) under continuous saline irrigation on each side of every mandible in a manner previously described (Deporter et al, 1986, 1990). An inter-implant distance of ~5 mm was left hopefully to encourage vertical rather horizontal bone loss. The tapered, press-fit implants with healing caps already in place were tapped to their fully seated positions using a surgical mallet and implant driver. Care was taken in doing all of this to ensure that the whole implant length was submerged in bone with only the healing cap at most proud of the alveolar crest. Afterwards, the implanted sites were irrigated with saline and the flaps repositioned and sutured so that the implants were fully submerged beneath the gingival flap during the initial healing phase.

During the first two weeks following implantation, all animals received the soft diet described. Anti inflammatory/ analgesic medication (Buprenorphine 0.8 ml/dog) and antibiotics (Baytril, Enrofloxacin 50 mg/ ml, Bayer Inc., Agriculture Division, Animal Health, Etobicoke, ON) were administrated as required during the first 48-72 hours post-operatively.

Re-entry procedure

Re-entry surgery had originally been planned after 4 weeks of initial healing of the implanted sites. However, as indicated, there was a delay in receiving the transgingival
components so that re-entry needed to be delayed until 8 weeks. A single mid-crestal incision was used to expose all three implants on each side of the mandible. After attaching an expanded healing abutment to each implant and securing it with its retaining screw, the flaps were repositioned and sutured around the abutments. The top of each screw was covered with self-curing methylmethacrylate to minimize the risk of screw loosening during function.

Establishment of Peri-implantitis

Braided cotton non-impregnated (Gingibraid, Van R Dental Products, Oxnard, CA, USA) or silk (4-0) ligatures (at the first session it was realized that there had been insufficient cotton ligature ordered and therefore silk ligatures had to be used for some of the implants) were tied tightly around each implant and forced into a position immediately apical to the gingival margin as described by others (Register & Burdick, 1975; Lindhe et al, 1992 Ericsson et al. 1996a) to combine the reaction induced by the presence of plaque with a mechanical disruption of the soft tissue seal. Knots in the ligatures were secured with Histoacryl (B. Braun Surgical GmbH, D-34209, Melsungen) glue to prevent loosening of the ligatures.

Bone loss in response to the ligatures was monitored by means of periodic radiographic examination as described below. For the entire period of ligature-induced disease, the animals received a soft diet (as described) further to encourage plaque accumulation and microbiological samples were taken (at baseline and at each change of ligature) further to monitor the development of disease. The ligatures were changed every 3 weeks and maintained for 6 weeks in two dogs and for 8 weeks in the remaining three. After the first change, all ligatures used were the cotton (Gingibraid) ones. Immediately following final ligature removal in each dog, the hygienic procedures described above were reinstated in order to improve the state of the peri-implant mucosal tissues prior to the rescue surgical procedures. As well, one week before this surgery the dogs were administered (orally) systemic antibiotics (Amoxicillin, 250 mg and Metronidazole 500 mg) twice daily as this
regimen is known to reduce pathogens typical of experimental peri-implantitis (Ericsson et al, 1996). This regimen was continued for 2 weeks after surgery as well.

The "Rescue" Procedures

Rescue surgery in each case was intended to be performed two weeks after the ligatures had been removed. This was possible in the first two dogs that had the ligatures in place for 6 weeks. However, scheduling problems resulted in the rescue surgeries being done four weeks after ligature removal in the remaining three dogs.

Two different "rescue" procedures were used based on the principles of guided bone regeneration. Both approaches were original because of the materials used and because it was the first time that an attempt was made to treat peri-implantitis around porous-surfaced dental implants. Rescue attempts around the implants on one side of the mandible employed Dynagraft Putty (demineralized, freeze-dried canine bone in a synthetic carrier) alone while on the contralateral side of the mandible; this graft material was further covered with Capset (medical grade calcium sulfate) as a barrier material.

For all rescue procedures a midcrestal incision was made in the peri-implant gingival tissues to permit elevation of buccal and lingual full-thickness flaps and exposure of the bone surrounding all implants. The healing abutments were removed and replaced with sterile (low profile) healing caps. Using curettes all granulation tissue was removed from the peri-implant bony defects that had resulted from the experimental disease. After saline irrigation of the curetted sites, all implant surfaces (smooth or porous) no longer buried in bone were decontaminated using a stiff brush and an aqueous solution of citric acid (Register & Burdick, 1975). To do this, the brush was first washed in sterile saline and then dipped in the acid and used to scrub the surfaces of one of the affected implants for about 10 seconds before repeating this sequence. The aim was to constantly replenish the brush with fresh acid and to continue scrubbing the surfaces of the three implants in the surgical site for 5 minutes. Afterwards, the surgical site and implants were washed extensively with sterile saline before proceeding further.
As indicated, two different grafting procedures were employed. On one side of the mandible, a graft of canine demineralized, freeze-dried bone matrix (35% by weight) dispersed in an aqueous poloxamer was used alone. This material was provided as a courtesy by GenSci OrthoBiologics (Irvine, CA). It was used to completely cover the exposed and acid-decontaminated porous surface of all implants before repositioning and sutureing the soft tissue flap. On the contralateral side, the acid-decontaminated implant surfaces were covered with the same graft material which was then itself covered with a calcium sulfate barrier (Capset, Swiss NF Metal Inc., Lifecore Biomedical, Chaska, MN, U.S.A.). Capset is a premeasured formulation of medical grade calcium sulfate (plaster of Paris) powder and an accelerating diluent solution. The powder and the diluent are combined to form a paste that hardens into a hard resorbable barrier within 4 minutes of mixing. During this 4-minute setting interval, the flaps were repositioned and sutured in an attempt to resubmerge all implants beneath a mucoperiosteal flap, creating a closed wound-healing environment. As indicated, the systemic antibiotics were continued for two weeks after the surgery.

Sutures were removed after 6 weeks in first two dogs and after 8 weeks in remaining three dogs, and afterwards the hygiene procedures were reinstituted three times a week and continued until the end of the study, i.e. three months after the rescue procedures. Care was taken to swab the tops of any healing caps which had not been completely submerged beneath mucoperiosteum or that became exposed during subsequent healing (12 out of 30).

**Microbiological assessment**

Microbiological samples were harvested from each implant at the time of ligature placement (baseline), each time that the ligatures were changed (weeks 3, 6, and in the case of three dogs, at week 8), just before the rescue surgery and for any implant with an exposed healing cap following the rescue procedure, just before sacrifice. *Porphyromonas gingivalis* was used as the marker microorganism as it is known to be
associated with periodontal disease and peri-implantitis in humans and animals. To collect the samples, after removal of gross supragingival plaque with water and a toothbrush, three fine paper points (one each on the mesio-buccal, mid-buccal and disto-buccal of the each abutment) per implant were inserted to the depth of the peri-implant sulcus or pocket and kept in place for 10 seconds (Mombelli et al, 1987; Leonhardt et al, 1992). The paper points were then removed and transported in 10 ml reduced transportation fluid (RTF) in sterile tubes, and assessed in a qualitative manner (Mombelli et al, 1987; Leonhardt et al, 1992) using immunofluorescence. The RTF with EDTA has the following chemical composition: 100 ml RTF comprises 7.5 ml of stock mineral salt solution #1 (prepared in the laboratory), 7.5 ml of stock mineral solution #2 (prepared in the laboratory), 1ml EDTA Na salt (0.1 M), 0.05 g Cysteine HCl, 0.5 ml Sodium carbonate, 83.5 ml distilled water. The solution is filter sterilized and refrigerated at 4 degrees Celsius and conditioned in an anaerobic environment 48h before use.

The samples were prepared for the immunofluorescence technique by placing 20 µl portions of the vortexed RTF in each well of 8-well slides. These were air-dried overnight, and the next day heat-fixed by passing them through a flame, rinsed with tap and distilled water and blow-dried with air. The anti-\textit{Porphyromonas gingivalis} antibody serum (1/50 dilution) was added, 10 µl/ well and the samples incubated at 37°C for 30 minutes. Thereafter, FITC-conjugated anti-rabbit antibody serum, 1/50 dilution, (BIOCAN Scientific Cat # 711-095-152) was placed on the samples, 10 µl/ well and again the slides were incubated at 37°C for 30 minutes. The incubation process allows for a better and faster outcome than leaving the samples overnight at the room temperature in the antigen-antibody reaction.

Reading of the results was performed by fluorescence microscopy in blue light (360-420 nm wavelengths) with a 63X magnification objective after mounting the slides with an anti-bleaching solution (DuPont® Chemical Solutions Enterprise) to prevent the fading of the immunofluorescent stain.
**Histological Preparation**

Twelve weeks after the rescue surgery, animals were killed with euthanasia chemicals (T-61, 5 ml/ dog) under general anesthesia with intravenous Pentobarbital. Bone biopsies containing the implant specimens were harvested and fixed in a mixture of formalin/ methanol/ water (1/1/1.5) for 1 week and thereafter dehydrated with ethanol (70-95-100%) in two changes per week for 2 weeks and embedded in polymethylmethacrylate (Osteobid) for 3 weeks. Four blocks were prepared for every sample: mesio-buccal, mesio-lingual, disto-buccal and disto-lingual. The faces of these blocks were stained with toluidine blue and light green for backscattered SEM assessment (see Appendix). Eight sections (150 μm thick) were produced, one from each face of each block: buccal-distal; distal-buccal; distal-lingual; lingual-distal; lingual-mesial; mesial-lingual; mesial-buccal; buccal-mesial and stained with toluidine blue and/or van Gieson (picro-fuchsin) for transmitted light microscopy (Figure 2).

**Figure 2.** – Schematic illustration of the sectioning technique which demonstrates how the sections are produced: 1 buccal-distal; 2 – distal buccal; 3- distal-lingual; 4- lingual-distal; 5- lingual-mesial; 6- mesial lingual; 7- mesial buccal; 8- buccal-mesial
Methods of Analysis

Radiographs

Radiographs were taken with the animals under general anesthesia as outlined above at the time of the ligature placement (week zero) and at each time the ligatures were changed (i.e. after weeks 3, 6, and in the case of three dogs, week 8) with the aim to monitor peri-implant marginal bone loss (saucerization). For each mandible of each animal, a custom-made stainless steel film holder was screwed into the middle implant after removal of its abutment. A portable x-ray machine (GENDEX apparatus model 4519 105 0077 G1, GENDEX CORP., Des Plains, Illinois, USA) was used at sensitivity setting of 5, 70 kVp and an exposure time of 0.50 seconds. The films used were KODAK ultra-speed D, size 2 films (EASTMAN KODAK Co., Rochester, NY, USA). All films were manually processed using KODAK GBX developer and fixer (CAT 190-1859) after appropriate dilution at 20° C and with standardized technique, i.e. 4.5 minutes in developer with no agitation, 30 seconds washed in water with gentle agitation, 10 minutes in fixer with no agitation, 30 minutes in running water.

Histological assessment

Blocks were examined using backscatter scanning electron microscopy (BSEM) to determine the bone-implant relationship, the level of the marginal bone, the original defect area and the degree of the bone regeneration. The measurements on BSEM micrographs performed using Sigma Scan Pro 5 computer program are listed below and illustrated in Figures 3A1, 3A2, 3B1, 3B2 and 3C.

Sections from the faces of the blocks were assessed using transmission light microscopy in order to confirm the correct delineation done with BSEM of the old and new bone, to characterize the architecture of the tissues surrounding the implant, to identify remnants of the graft materials and presence of inflammation.
Parameters measured using BSEM and Sigma Scan Pro 5 computer program included:

(A) The straight-line height of the implant surface exposed in the original peri-implantitis defect using the smooth-porous junction as the reference point (i.e. from the smooth-porous junction to the bottom of the defect)

(B) The straight-line height of regenerated bone from the bottom of the original defect to the most coronal point of new bone formation in contact with the porous surface

(C) The maximal length of the outermost layer of particles of porous implant surface exposed in the original defect and available for contact with new bone

(D) The maximal length available as in (C) but in relation to porous implant surface not involved in the original defect, i.e. non-defect associated surface

(E) The length of the outermost layer of particles now in contact with bone within the original defect area (Absolute Contact Length, ACL-d)

(F) The same as (E) but for the non-defect area (ACL-nd)

(G) The area of beads of the porous surface within the original defect

(H) The same as (G) but for the porous surface related to the non-defect-associated segment of the implant

(I) The area of bone occupying the implant porosity in relation to the initial bone defect

(J) The same as (I) but for the non-defect-related segment of the implant

(K) The area within the initial bone defect adjacent to porous implant surface (and not including (I)) now occupied by new bone

(L) The area within the original bone defect adjacent to the porous implant surface now occupied by "others" (i.e., soft tissues, empty spaces)

(M) The area within the original bone defect and adjacent to the smooth collar region now occupied by bone, using the top of the implant as the reference point

(N) The area within the original bone defect and adjacent to the smooth collar region now occupied by "others", using the top of the implant as the reference point

(O) The area enclosed by the implant solid core, a line drawn through the outermost part of the smooth collar and parallel to the solid core of the fixture, the bottom of the defect and the point of the junction of the smooth collar and porous surface
Area enclosed by the implant solid core, a line drawn through the outermost part of the smooth collar and parallel to the solid core, the bottom of the defect and the bottom of the fixture.

Further combinations of these measurements were calculated as follows:

1) The Regenerated Bone Height (RBH) or re-osseointegration: fraction $(B)/(A)$
2) The Contact Length Fraction for the defect area (CLFd) being the fraction $(E)/(C)$
3) The Contact Length Fraction for the non-defect area (CLFnd) being the fraction $(F)/(D)$
4) The Bone Ingrowth Fraction for the defect area (BIFd), that being the fraction $(J)/(O-G)$
5) The Bone Ingrowth Fraction for the non-defect area (BIFnd), $(J)/(P-H)$
6) The area of the initial peri-implantitis bone defect now filled with bone: $(I+K+M)$
7) The area of the initial peri-implantitis bone defect now filled with “others”: $(L+N)$
8) The area of the original peri-implantitis bone defect using the top of the implant as reference point $(K+M+I+L+N)$
9) The “bone gain” $(B)$
Figure 3A1. - Backscatter-SEM image (magnification X60) of an implant from Dynagraft only treatment group, which depicts the measurement techniques; linear measurements using Sigma Scan Pro5 computer program
- Red dotted line: delineation between old bone (OB) and new bone (NB)
- Blue: the straight-line height of the implant surface exposed in the original peri-implantitis defect using the smooth-porous junction as the reference point (i.e. from the smooth-porous junction to the bottom of the defect): A
- Orange: “bone gain” the straight line height of regenerated bone from the bottom of the original defect to the most coronal point of new bone formation in contact with the porous surface: B
- Red: the maximal length of the outermost layer of particles of porous implant surface exposed in the original defect (i.e., defect) or in relation to porous implant surface not involved in the original defect (i.e., non-defect) and available for contact with new bone: C, D
- Green: the length of the outermost layer of particles now in contact with bone within the original defect area (Absolute Contact Length, ACLd) or in relation to porous implant surface not involved in the original defect (Absolute Contact Length, ACLnd): E, F
Figure 3A2. - Backscatter-SEM image (magnification X60) of an implant from Dynagraft+Capset treatment group, which depicts the measurement techniques; linear measurements using Sigma Scan Pro5 computer program.
- Red dotted line: delineation between old bone (OB) and new bone (NB)
- Blue: the straight-line height of the implant surface exposed in the original peri-implantitis defect using the smooth-porous junction as the reference point (i.e. from the smooth-porous junction to the bottom of the defect): A
- Orange: "bone gain" the straight line height of regenerated bone from the bottom of the original defect to the most coronal point of new bone formation in contact with the porous surface: B
- Red: the maximal length of the outermost layer of particles of porous implant surface exposed in the original defect (i.e., defect) or in relation to porous implant surface not involved in the original defect (i.e., non-defect) and available for contact with new bone: C, D
- Green: the length of the outermost layer of particles now in contact with bone within the original defect area (Absolute Contact Length, ACL-d) or in relation to porous implant surface not involved in the original defect (Absolute Contact Length, ACL-nd): E, F
Figure 3B1 - Backscatter-SEM image (magnification X60) of an implant from Dynagraft only treatment group, which depicts the measurement techniques; area measurements using Sigma Scan Pro5 computer program
- Red: the area of bone occupying the implant porosity in relation to the initial bone defect or to the non-defect area: I, J
- Yellow: the area within the initial bone defect adjacent to porous implant surface and to the smooth implant surface now occupied by new bone ("area bone"): K, M
- Green: the area within the initial bone defect adjacent to porous implant surface and to the smooth implant surface now occupied by tissues, other than bone ("area other"): L, N
Figure 3B2 - Backscatter-SEM image (magnification X60) of an implant from Dynagraft+Capset treatment group, which depicts the measurement techniques; area measurements using Sigma Scan Pro5 computer program.

- Red: the area of bone occupying the implant porosity in relation to the initial bone defect or to the non-defect area: I, J
- Yellow: the area within the initial bone defect adjacent to porous implant surface and to the smooth implant surface now occupied by new bone ("area bone"): K, M
- Green: the area within the initial bone defect adjacent to porous implant surface and to the smooth implant surface now occupied by tissues, other than bone ("area other"): L, N
Figure 3C. - Backscatter-SEM image (magnification X60) of an implant from Dynagraft only treatment group, which depicts the measurement techniques; area measurements using Sigma Scan Pro5 computer program (similar for Dynagraft + Capset treatment group)

-Red dotted line: delimitation between old bone (OB) and new bone (NB)
-Blue: the area enclosed by the implant solid core, a line drawn through the outermost part of the smooth collar and parallel to the solid core of the fixture, the bottom of the defect and the point of the junction of the smooth collar and porous surface: O
-Blue: the area enclosed by the implant solid core, a line drawn through the outermost part of the smooth collar and parallel to the solid core, the bottom of the defect and the bottom of the fixture:P
Statistical Treatment of the Data

Before undertaking the study a sample size calculation was performed to determine the number of the implants to use, and it was estimated that for statistically significant results to be achieved ($p<0.05$), at least 10 sites were needed for each of the two surgical procedures of treatment.

The sample size calculation is given by:

$$n = \frac{[(u + v)^2 \times (\sigma_1^2 + \sigma_2^2)]}{(\mu_1 - \mu_2)^2}$$

$$n = \frac{[(1.28 + 1.96)2 \times (2^2 + 2^2)]}{3^2} = 9.33$$

$n$: minimum sample size to achieve statistically significant results

$u$: one-sided percentage point of the normal distribution corresponding to 100% minus the power

$v$: percentage point of the normal distribution corresponding to the two-sided significance level

$\sigma_1, \sigma_2$: standard deviation

$\mu_1, \mu_2$: mean

Therefore, for the study it was decided to use 5 dogs, 6 implants per dog and 8 sections per implant giving a value for $n=240$. Eight sections were lost because of technical difficulties, and as they were paired for paired T-test assessment, their matches were also excluded from the calculations. The final sample size on which statistics were performed was therefore $n=224$. The data resulting from BSEM assessment were statistically analyzed using paired t-test and ANCOVA to detect differences, if any, between the two treatment approaches.
V. Results

All thirty implant fixtures achieved osseointegration in the 8-week healing interval provided and received their transgingival abutments without complication. Placement and maintenance of the ligatures took place without problems, and the peri-implant mucosal tissues quickly showed inflammatory changes in response to the withdrawal of oral hygiene and the prescribed soft diet.

**Peri-implantitis Phase**

**Clinical assessment**

After ligature placement the animals were examined at weeks 3, 6 and 8 (in the case of three dogs). Clinically, gross plaque adherence to the abutments (Figure 4), severely inflamed gingival tissues and bleeding on gentle manipulation were observed at all of these examinations. None of the implants exhibited mobility, i.e. loss of osseointegration, during any of the manipulations associated with these examinations including use of the radiographic film holder.

**Radiographic exam**

Standardized periapical radiographs were used to monitor crestal bone loss in response to the ligatures and to determine the time at which ligatures should be removed. There was variation amongst dogs and amongst implants, 6 to 8 weeks after placement of the cotton ligatures bone loss had occurred in relation to the majority of the implants so as to denude porous surfaces.

The pattern of the bone resorption was mostly horizontal with the exception of the mesial surfaces of the most-anterior implants and distal surfaces of the most-distal implants where vertical bone resorption occurred, i.e. bone defects had walls of varying height.
Figures 5A and 5B illustrate the crestal bone level and the pattern of bone resorption at the baseline and at the time when the ligatures were removed.

**Microbiological assessment**

Immunofluorescent staining was used to identify the presence of *P. gingivalis* (PG) as an indicator of established peri-implantitis. Samples collected at baseline (time when the ligatures were first placed) demonstrated that none of the sites harbored this microorganism. Initially, an attempt was made to use the criteria of positivity described by Mombelli (1987), that is, the presence of at least 20 positively-stained microorganisms in each of 20 microscopic fields examined (in other words, at least 400 positively-stained bacteria in each specimen). This criterion, however, could not be met as none of the samples had all 20 microscopic fields with the required minimum 20 organisms. Therefore, it was necessary to use a qualitative method only, i.e. a “present” vs. “absent” type of answer. At the time when the ligatures were removed, PG was identified at all peri-implantitis site using this qualitative assessment (Figure 6).

As indicated in the “Materials and Methods” section, 12 of the 30 fixtures were not successfully re-submerged following the rescue surgical procedures and their healing caps remained partially or completely exposed to the oral environment until the end of the study. For these, a final microbiological assessment was performed immediately before animal sacrifice, and none to very few PG were identified in these samples.
Figure 4.

Gross plaque accumulation facilitated by the ligature presence
Figure 5A
Standard periapical radiographs taken:
A- At the time of ligature placement (arrows indicate the initial bone level)
B- At the time of ligature removal; horizontal bone resorption can be detected (arrows indicate the bone level)
Figure 5B
Standard periapical radiographs taken:
A- At the time of ligature placement (arrows indicate the initial bone level)
B- At the time of ligature removal; vertical bone resorption can be detected (arrows indicate the bone level)
Figure 6.
Immunofluorescent staining of the *P.gingivalis* as detected at all peri-implantitis sites when the ligatures were removed
Figure 7.
Peri-implant mucosa: inflammation caused by gross plaque accumulation is reduced by hygienic procedures and antibiotics administration; photograph taken before "rescue" surgery
Figure 8.
Porous-surfac ed segments no longer submerged into the adjacent crestal bone; bone pattern resorption observed on radiographs was confirmed during surgery (arrows). Photograph taken during surgery, after debridement.
Figure 9.
A- Dynagraft Putty placed over the crestal bone defects (arrows)
B- Capset placed on top of the graft material (arrow)
Rescue Phase

As described, for two weeks prior to rescue surgery, thrice-weekly oral hygiene procedures were re-instituted so that by the time of the proposed rescue surgery the soft tissues adjacent to implants could be described as apparently healthy looking with no visible signs of inflammation (see Figure 7). After removal of the abutments when buccal and lingual full-thickness flaps were raised and thorough debridement was performed, the exposure of the bone surrounding all implants allowed us to observe the bone resorption pattern which was mostly horizontal with vertical walls at the mesial aspect of the mesial implants and distal aspect of the distal implants, as assessed on the radiographs. Crater-like, circumferential bone resorption of about 1 mm deep, right around the fixtures could be described for some fixtures (Figure 8).

On one mandibular side (chosen by the flip of a coin) the Dynagraft Putty was used alone. On the contralateral side, the same graft material was used combined with a resorbable barrier, Capset (Figure 9).

Outcomes of the Rescue Treatments

Assessment with BSEM

As described (see “Materials and Methods”), descriptive statistics and paired t-tests were performed on all parameters measured by BSEM to detect differences between the two treatment approaches. ANCOVA, which introduces covariates in the model, was used for the outcome variable “area bone”, controlling for the covariate “area defect”.

The sample size on which statistics were performed was “n”=112 for each treatment group as 16 out of 240 sections were lost or excluded from the analysis. Table 5 displays descriptive statistics for all of the parameters measured. It can be seen that the RBH, ACL-nd, CLF-d, BIF-d and BIF-nd, “area bone” and “bone gain” values all appear better for the combined treatment (Dynagraft +Capset). For ACL-d, CLF-nd and “area other”, the Dynagraft only treatment group apparently performed better (bigger values stand for
better outcome). The mean values for the parameter “area defect” were identical for the two treatment groups.

Table 5 - Descriptive statistics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>Mean (Dynagraft only)</th>
<th>SE mean</th>
<th>Mean (Dynagraft+ Capset)</th>
<th>SE mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBH</td>
<td>112</td>
<td>0.32</td>
<td>0.299</td>
<td>0.48</td>
<td>0.389</td>
</tr>
<tr>
<td>Bone gain (mm)</td>
<td>112</td>
<td>0.60</td>
<td>0.58</td>
<td>0.89</td>
<td>0.881</td>
</tr>
<tr>
<td>ACL-d (mm)</td>
<td>112</td>
<td>1.17</td>
<td>0.13</td>
<td>1.06</td>
<td>0.10</td>
</tr>
<tr>
<td>ACL-nd (mm)</td>
<td>112</td>
<td>2.32</td>
<td>0.11</td>
<td>2.74</td>
<td>0.16</td>
</tr>
<tr>
<td>CLF-d</td>
<td>112</td>
<td>0.17</td>
<td>0.018</td>
<td>0.20</td>
<td>0.021</td>
</tr>
<tr>
<td>CLF-nd</td>
<td>112</td>
<td>0.35</td>
<td>0.017</td>
<td>0.33</td>
<td>0.017</td>
</tr>
<tr>
<td>BIF-d</td>
<td>112</td>
<td>0.18</td>
<td>0.019</td>
<td>0.24</td>
<td>0.023</td>
</tr>
<tr>
<td>BIF-nd</td>
<td>112</td>
<td>0.38</td>
<td>0.018</td>
<td>0.42</td>
<td>0.020</td>
</tr>
<tr>
<td>Area bone (mm²)</td>
<td>112</td>
<td>0.98</td>
<td>0.072</td>
<td>1.09</td>
<td>0.069</td>
</tr>
<tr>
<td>Area other (mm²)</td>
<td>112</td>
<td>2.08</td>
<td>0.093</td>
<td>1.97</td>
<td>0.099</td>
</tr>
<tr>
<td>Area defect (mm²)</td>
<td>112</td>
<td>3.08</td>
<td>0.075</td>
<td>3.08</td>
<td>0.099</td>
</tr>
</tbody>
</table>

SE = standard error.
RBH = regained bone height (bone gain/ initial bone defect straight line measurement, i.e., re-osseointegration);
ACL-d = absolute contact length, defect area;
ACL-nd = absolute contact length, non-defect area;
CLF-d = contact length fraction, defect area;
CLF-nd = contact length fraction, non-defect area;
BIF-d = bone ingrowth fraction, defect area;
BIF-nd = bone ingrowth fraction, non-defect area;
Bone gain = linear measurement of the bone height from bottom of the defect-to-most coronal point of the new bone.

The paired t-test (bivariate analysis) was used to detect differences if any between the two treatment groups and to establish if these differences were statistically significant. The paired design was chosen because the same graft material was used in both treatment

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approaches and this was the matching criterion. A given section from one treatment group was paired with its corresponding one from the other treatment group.

Table 6 shows the results of these tests and it can be concluded that there are statistically significant differences (p<0.05) between the two treatment approaches for the parameters RBH, ACL-nd, BIF-d and “bone gain” the differences favoring the Dynagraft +Capset treatment group.

Table 6 - Paired T-Test Results for the Two Treatments

<table>
<thead>
<tr>
<th>Paired parameters (Dynagraft only vs. Dynagraft+Capset)</th>
<th>Mean difference</th>
<th>SE</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair 1 RBH</td>
<td>-0.16</td>
<td>0.042</td>
<td>0.004*</td>
</tr>
<tr>
<td>Pair 2 “bone gain”</td>
<td>-0.28</td>
<td>0.097</td>
<td>0.004*</td>
</tr>
<tr>
<td>Pair 3 ACL-d (mm)</td>
<td>0.10</td>
<td>0.13</td>
<td>0.401</td>
</tr>
<tr>
<td>Pair 4 ACL-nd (mm)</td>
<td>-0.42</td>
<td>0.18</td>
<td>0.021*</td>
</tr>
<tr>
<td>Pair 5 CLF-d</td>
<td>-0.028</td>
<td>0.02</td>
<td>0.156</td>
</tr>
<tr>
<td>Pair 6 CLF-nd</td>
<td>0.014</td>
<td>0.023</td>
<td>0.552</td>
</tr>
<tr>
<td>Pair 7 BIF-d</td>
<td>-0.062</td>
<td>0.024</td>
<td>0.013*</td>
</tr>
<tr>
<td>Pair 8 BIF-nd</td>
<td>-0.037</td>
<td>0.025</td>
<td>0.143</td>
</tr>
<tr>
<td>Pair 9 “area bone” (mm²)</td>
<td>-0.11</td>
<td>0.10</td>
<td>0.283</td>
</tr>
<tr>
<td>Pair 10 “area other” (mm²)</td>
<td>0.11</td>
<td>0.12</td>
<td>0.352</td>
</tr>
<tr>
<td>Pair 11 “area defect” (mm²)</td>
<td>-0.002</td>
<td>0.11</td>
<td>0.983</td>
</tr>
</tbody>
</table>

SE: standard deviation.
* Statistically significant, for p<0.05
The mean difference is calculated by subtracting the mean of the measured parameter in the Dynagraft +Capset treatment group from the mean of the same parameter in the Dynagraft only treatment group. The negative values of the “Mean difference” indicate that the Dynagraft +Capset treatment group performed better.

Interesting results were also obtained when the paired design was used within each implant, that is when matching pairs were constructed to compare the segments of implant that were exposed (the segment “re-osseointegrated” after rescue) or not exposed (the segment where initial integration presumably had been maintained) to the experimental peri-implantitis, and how they differed in relation to the treatment procedures. These results can be seen in Table 7 which indicates that there are significant
differences (p<0.05) for all tested parameters in the defect-related segments of the implants compared with non-defect segments, the latter always having the greater values. These observed differences may be related to the fact that the defect areas were assessed 3 months after treatment while the non-defect areas had been osseointegrated from the beginning of the study (about 8 months in total) or, the previously infected and “rescued” implant surfaces might not be as friendly as the clean, sterile ones, to cell attachment.

Table 7: Defect vs. non-defect Associated Segments of Implant Surface: Paired T-Test Results

<table>
<thead>
<tr>
<th>Pairs (defect vs. non-defect)</th>
<th>Mean difference</th>
<th>SE</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pair1-ACL (mm) Dynagraft only</td>
<td>-1.15</td>
<td>0.14</td>
<td>0.000*</td>
</tr>
<tr>
<td>Pair2-CLF Dynagraft only</td>
<td>-0.17</td>
<td>0.022</td>
<td>0.000*</td>
</tr>
<tr>
<td>Pair3-BIF Dynagraft only</td>
<td>-0.20</td>
<td>0.021</td>
<td>0.000*</td>
</tr>
<tr>
<td>Pair4-ACL (mm) Dynagraft+Capset</td>
<td>-0.17</td>
<td>0.16</td>
<td>0.000*</td>
</tr>
<tr>
<td>Pair5-CLF Dynagraft+Capset</td>
<td>-0.13</td>
<td>0.24</td>
<td>0.000*</td>
</tr>
<tr>
<td>Pair6-BIF Dynagraft+Capset</td>
<td>-0.18</td>
<td>0.029</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

SE: standard deviation.
* : Statistically significant, for p<0.05
The “Mean difference” is calculated by subtracting the mean of the measured parameter in the non-defect area from the mean of the same parameter in the defect areas. The negative values of the mean difference indicate that the non-defect areas showed better results.

Results from ANCOVA

Between-subjects factors that were tested included: treatment group (i.e. Dynagraft+ Capset vs. Dynagraft only), implant position (i.e. mesial, central, distal) and section (distobuccal, mesiobuccal, buccal, lingual, distolingual, mesiolingual). The dependent (outcome) variable was “area bone” and it was calculated by controlling for the covariate...
“area defect”. Table 8 shows all between-subjects interactions while Table 9 displays only the statistical significant ones.

Only the AREAD (“area defect”) and SITE (section) have a statistically significant effect \( p<0.05 \) on the dependent variable “area bone”. Although the treatment (TREAT) has no significant effect \( (p>0.05) \), it was kept in the model because we wanted to detect differences, if any, between the two treatment approaches. The model was further corrected by removing the factors with no significance. The new results for Between-Subjects effects, for dependent variable area bone and controlling for the covariate area defect are presented below. Again, AREAD (area defect) and SITE (section) had statistically significant effect \( (p<0.05) \).

Estimated marginal means for the dependent variable “area bone” after controlling for the covariate “area defect” and evaluating the treatment groups are presented in the Table 10. Overlapping of the confidence interval values i.e. upper boundary for Dynagraft only and lower boundary for Dynagraft + Capset, implies no statistically significant difference when comparing the area of the new bone resulting from the two treatment approaches.

Table 8 ANCOVA Test of Between-Subjects Effects

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected model</td>
<td>29.931 [^a]</td>
<td>48</td>
<td>0.624</td>
<td>1.139</td>
<td>0.270</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.095</td>
<td>1</td>
<td>0.095</td>
<td>0.173</td>
<td>0.678</td>
</tr>
<tr>
<td>AREAD</td>
<td>14.24</td>
<td>1</td>
<td>14.27</td>
<td>25.998</td>
<td>0.000*</td>
</tr>
<tr>
<td>SITE</td>
<td>9.53</td>
<td>7</td>
<td>1.362</td>
<td>2.487</td>
<td>0.019*</td>
</tr>
<tr>
<td>TREAT</td>
<td>0.69</td>
<td>1</td>
<td>0.697</td>
<td>1.272</td>
<td>0.261</td>
</tr>
<tr>
<td>IMPLANT</td>
<td>0.088</td>
<td>2</td>
<td>0.044</td>
<td>0.081</td>
<td>0.922</td>
</tr>
<tr>
<td>SITE*TREAT</td>
<td>1.49</td>
<td>7</td>
<td>0.213</td>
<td>0.389</td>
<td>0.908</td>
</tr>
<tr>
<td>SITE*IMPLANT</td>
<td>3.75</td>
<td>14</td>
<td>0.268</td>
<td>0.489</td>
<td>0.937</td>
</tr>
<tr>
<td>TREAT*IMPLANT</td>
<td>2.88</td>
<td>2</td>
<td>1.442</td>
<td>2.633</td>
<td>0.075</td>
</tr>
<tr>
<td>SITE<em>TREAT</em>IMPLANT</td>
<td>2.66</td>
<td>14</td>
<td>0.190</td>
<td>0.347</td>
<td>0.986</td>
</tr>
<tr>
<td>Error</td>
<td>95.84</td>
<td>175</td>
<td>0.548</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>369.63</td>
<td>224</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected total</td>
<td>125.77</td>
<td>223</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\[^a\] R Squared= 0.238 (Adjusted R Squared = 0.029)

* Statistically significant for \( p<0.05 \)
Table 9 ANCOVA Between-Subjects Effects – Corrected model

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected model</td>
<td>19.370&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9</td>
<td>2.152</td>
<td>4.329</td>
<td>0.000*</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.312</td>
<td>1</td>
<td>0.312</td>
<td>0.627</td>
<td>0.429</td>
</tr>
<tr>
<td>TREAT</td>
<td>0.701</td>
<td>1</td>
<td>0.701</td>
<td>1.411</td>
<td>0.236</td>
</tr>
<tr>
<td>SITE</td>
<td>9.094</td>
<td>7</td>
<td>1.299</td>
<td>2.613</td>
<td>0.013*</td>
</tr>
<tr>
<td>AREAD</td>
<td>14.485</td>
<td>1</td>
<td>14.485</td>
<td>29.134</td>
<td>0.000*</td>
</tr>
<tr>
<td>Error</td>
<td>106.397</td>
<td>214</td>
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<td>Total</td>
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<tr>
<td>Corrected total</td>
<td>125.767</td>
<td>223</td>
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</tr>
</tbody>
</table>

a. R Squared = 0.154 (Adjusted R Squared = 0.118)

Table 10 ANCOVA - Estimated marginal means

Dependent variable: "area bone"

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Adjusted means</th>
<th>95% Confidence interval</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower bound</td>
<td>Upper bound</td>
</tr>
<tr>
<td>Dynagraft only</td>
<td>0.987&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.856</td>
<td>1.119</td>
</tr>
<tr>
<td>Dynagraft + Capset</td>
<td>1.099&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.968</td>
<td>1.231</td>
</tr>
</tbody>
</table>

a. Evaluated at covariates appeared in the model: area defect = 3.08378

Further, we have tested if the fact that the fixtures were submerged after the rescue surgery would affect the treatment outcome. Table 11 presents the results from descriptive statistics from submerged fixtures only, from both treatment groups. The sections from the non-submerged fixtures and their corresponding ones were removed from the analysis, as the paired t-test was again performed. Paired t-test was performed for a sample size with \( n_1 = 32 \) for each treatment group. The results are presented in Table 12.
Table 11 - Descriptive statistics: Results for the Two Treatments in Submerged Implants

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Parameter</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBH</td>
<td>32</td>
<td>0.27</td>
<td>0.23</td>
<td>RBH</td>
<td>32</td>
<td>0.46</td>
<td>0.42</td>
</tr>
<tr>
<td>Bone gain (mm)</td>
<td>32</td>
<td>0.45</td>
<td>0.42</td>
<td>Bone gain (mm)</td>
<td>32</td>
<td>0.84</td>
<td>0.88</td>
</tr>
<tr>
<td>BIF-d</td>
<td>32</td>
<td>0.20</td>
<td>0.20</td>
<td>BIF-d</td>
<td>32</td>
<td>0.28</td>
<td>0.28</td>
</tr>
<tr>
<td>Area bone (mm²)</td>
<td>32</td>
<td>0.74</td>
<td>0.46</td>
<td>Area bone (mm²)</td>
<td>32</td>
<td>1.28</td>
<td>0.85</td>
</tr>
</tbody>
</table>

SD: standard deviation  
RBH = regained bone height (bone gain/initial bone defect straight line measurement, i.e., re-osseointegration);  
Bone gain = linear measurement of the bone height from bottom of the defect-to-most coronal point of the new bone.  
BIF-d = bone ingrowth fraction, defect area

Table 12 - Paired T-Test Results for the Two Treatments in Submerged Implants

<table>
<thead>
<tr>
<th>Paired parameters</th>
<th>Mean difference</th>
<th>SE</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Dynagraft only vs. Dynagraf+Capset)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pair 1 RBH</td>
<td>-0.19</td>
<td>0.091</td>
<td>0.039*</td>
</tr>
<tr>
<td>Pair 2 “bone gain”</td>
<td>-0.39</td>
<td>0.156</td>
<td>0.018*</td>
</tr>
<tr>
<td>Pair 3 ACL-d (mm)</td>
<td>-0.22</td>
<td>0.227</td>
<td>0.324</td>
</tr>
<tr>
<td>Pair 4 ACL-nd (mm)</td>
<td>0.24</td>
<td>0.333</td>
<td>0.465</td>
</tr>
<tr>
<td>Pair 5 CLF-d</td>
<td>-0.04</td>
<td>0.043</td>
<td>0.287</td>
</tr>
<tr>
<td>Pair 6 CLF-nd</td>
<td>0.00</td>
<td>0.047</td>
<td>0.842</td>
</tr>
<tr>
<td>Pair 7 BIF-d</td>
<td>-0.083</td>
<td>0.060</td>
<td>0.179</td>
</tr>
<tr>
<td>Pair 8 BIF-nd</td>
<td>-0.03</td>
<td>0.046</td>
<td>0.450</td>
</tr>
<tr>
<td>Pair 9 “area bone” (mm²)</td>
<td>-0.53</td>
<td>0.125</td>
<td>0.000*</td>
</tr>
<tr>
<td>Pair 10 “area other” (mm²)</td>
<td>-0.118</td>
<td>0.225</td>
<td>0.605</td>
</tr>
<tr>
<td>Pair 11 “area defect” (mm²)</td>
<td>-0.588</td>
<td>0.261</td>
<td>0.032</td>
</tr>
</tbody>
</table>

SE: standard deviation.  
* Statistically significant, for p<0.05  
The mean difference is calculated by subtracting the mean of the measured parameter in the Dynagraft +Capset treatment group from the mean of the same parameter in the Dynagraft only treatment group. The negative values of the “Mean difference” indicate that the Dynagraft +Capset treatment group performed better.
Parameters RBH, "area bone", "bone gain" and "area bone" presented significantly better values (p<0.05) for the Dynagraft+Capset treatment group, when only the submerged implants are considered. As noted from Table 7, "bone gain" and RBH presented significantly better values for the Dynagraft + Capset treatment group when sections from all implants (submerged and exposed) were analyzed, while paired t-test failed to detect significant differences between the two treatment procedures for parameter "area bone". Parameter BIFd presented statistically significant better values for Dynagraft + Capset treatment group when data from all fixtures were analyzed. However, when only sections from submerged fixtures were taken into analysis, paired t-test failed to detect significant differences between the two treatment approaches, fact that suggests that submerging or not the implants after "rescue" surgery might not influence bone ingrowth capacity.

Results from ANCOVA- submerged fixtures

Between-subjects factors that were tested included: treatment group (i.e. Dynagraft+Capset vs. Dynagraft only), implant position (i.e. mesial, central, distal) and section (distobuccal, mesiobuccal, buccal, lingual, distolingual, mesiolingual). The dependent (outcome) variable was "area bone" and it was calculated by controlling for the covariate "area defect". Table 13 shows all between-subjects interactions while Table 14 displays only the statistical significant ones.
Table 13 - ANCOVA Test of Between-Subjects Effects

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected model</td>
<td>22.048</td>
<td>32</td>
<td>0.689</td>
<td>1.798</td>
<td>0.053</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.715</td>
<td>1</td>
<td>0.715</td>
<td>1.867</td>
<td>0.182</td>
</tr>
<tr>
<td>AREAD</td>
<td>5.621</td>
<td>1</td>
<td>5.621</td>
<td>14.699</td>
<td>0.001*</td>
</tr>
<tr>
<td>SITE</td>
<td>5.726</td>
<td>7</td>
<td>0.818</td>
<td>2.135</td>
<td>0.069</td>
</tr>
<tr>
<td>TREAT</td>
<td>0.006</td>
<td>1</td>
<td>0.006</td>
<td>0.017</td>
<td>0.898</td>
</tr>
<tr>
<td>IMPLANT</td>
<td>0.191</td>
<td>1</td>
<td>0.191</td>
<td>0.498</td>
<td>0.486</td>
</tr>
<tr>
<td>SITE*TREAT</td>
<td>2.219</td>
<td>7</td>
<td>0.317</td>
<td>0.827</td>
<td>0.573</td>
</tr>
<tr>
<td>SITE*IMPLANT</td>
<td>2.665</td>
<td>7</td>
<td>0.381</td>
<td>0.994</td>
<td>0.454</td>
</tr>
<tr>
<td>TREAT*IMPLANT</td>
<td>1.484</td>
<td>1</td>
<td>1.484</td>
<td>3.872</td>
<td>0.058</td>
</tr>
<tr>
<td>SITE<em>TREAT</em>IMPLANT</td>
<td>2.508</td>
<td>7</td>
<td>0.358</td>
<td>0.935</td>
<td>0.494</td>
</tr>
<tr>
<td>Error</td>
<td>11.879</td>
<td>31</td>
<td>0.383</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>99.853</td>
<td>64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected total</td>
<td>33.927</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared= 0.650 (Adjusted R Squared = 0.288)
* Statistically significant for p<0.05

From the Table 13 it can be seen that AREAD ("area defect") had significant effect (p<0.05) on the parameter "area bone" when controlling for "area defect". The model was further corrected by removing the factors with no significance. The new results for Between-Subjects effects, for dependent variable area bone and controlling for the covariate area defect are presented in Table 14 which shows that TREAT (treatment) and AREAD (area of the initial bone defect) significantly influenced the treatment outcome (p<0.05).

Estimated marginal means for the dependent variable "area bone" controlling for the covariate "area defect" and evaluating the treatment groups are presented in the Table 15. Overlapping of the confidence interval values i.e. upper boundary for Dynagraf only and lower boundary for Dynagraf + Capset, implies no statistically significant difference when comparing the area of the new bone resulting from the two treatment approaches.
Table 14 - ANCOVA Between-Subjects Effects –Corrected model

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected model</td>
<td>9.068</td>
<td>2</td>
<td>4.534</td>
<td>11.126</td>
<td>0.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.545</td>
<td>1</td>
<td>0.545</td>
<td>1.338</td>
<td>0.252</td>
</tr>
<tr>
<td>TREAT</td>
<td>2.193</td>
<td>1</td>
<td>2.193</td>
<td>5.381</td>
<td>0.024*</td>
</tr>
<tr>
<td>AREAD</td>
<td>4.481</td>
<td>1</td>
<td>4.481</td>
<td>10.996</td>
<td>0.002*</td>
</tr>
<tr>
<td>Error</td>
<td>24.859</td>
<td>61</td>
<td>0.408</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>99.853</td>
<td>64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected total</td>
<td>33.927</td>
<td>63</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = 0.267 (Adjusted R Squared = 0.243)

Table 15 ANCOVA - Estimated marginal means

Dependent variable: “area bone”

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Adjusted means</th>
<th>95% Confidence interval</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower bound</td>
<td>Upper bound</td>
</tr>
<tr>
<td>Dynagraft only</td>
<td>0.822</td>
<td>0.592</td>
<td>1.053</td>
</tr>
<tr>
<td>Dynagraft + Capset</td>
<td>1.207</td>
<td>0.977</td>
<td>1.438</td>
</tr>
</tbody>
</table>

a. Evaluated at covariates appeared in the model: area defect = 2.8940

Further analysis was performed to detect differences, if any for parameter “area bone-porous” as this area was calculated in relation with porous surface only (calculated as I + K), ignoring the area of the initial defect now filled with bone in relation with the machined collar and labeled “M” in our initial measurements.

Tables 16, 17 and 18 are presenting the results of descriptive statistics, paired t-test and ANCOVA for this new parameter (area bone-porous) for the whole sample (n= 112).
Table 16 Descriptive statistics: "area bone-porous" results for the two treatment approaches

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area bone-porous (mm$^2$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dynagraft only</td>
<td>112</td>
<td>0.83</td>
<td>0.58</td>
</tr>
<tr>
<td>Area bone-porous (mm$^2$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dynagraft + Capset</td>
<td>112</td>
<td>0.90</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Table 17 Paired T-Test: "area bone-porous" results for the two treatment approaches

<table>
<thead>
<tr>
<th>Paired parameters</th>
<th>Mean difference</th>
<th>SE</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Dynagraft only vs. Dynagraft+Capset)</td>
<td>-0.069</td>
<td>0.080</td>
<td>0.390</td>
</tr>
</tbody>
</table>

SE: standard deviation.
* Statistically significant, for p<0.05

The mean difference is calculated by subtracting the mean of the measured parameter in the Dynagraft +Capset treatment group from the mean of the same parameter in the Dynagraft only treatment group. The negative values of the "Mean difference" indicate that the Dynagraft +Capset treatment group performed better.

Table 18 ANCOVA: "area bone-porous" results for the two treatment approaches

Estimated marginal means

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Adjusted means</th>
<th>95% Confidence interval Lower bound</th>
<th>95% Confidence interval Upper bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dynagraft only</td>
<td>0.827$^a$</td>
<td>0.727</td>
<td>0.926</td>
</tr>
<tr>
<td>Dynagraft + Capset</td>
<td>0.896$^a$</td>
<td>0.796</td>
<td>0.995</td>
</tr>
</tbody>
</table>

a. Evaluated at covariates appeared in the model: area defect= 3.0838

It can be noted (Table 16) that even if the descriptive statistics appeared to favor the combined treatment approach, both paired t-test and ANCOVA controlling for the covariate "area defect" failed to reveal significant differences between the two treatment groups for the parameter "area bone-porous".

82
Again from the analysis were removed sections from the non-submerged fixtures and their corresponding ones, and the same analyses was performed on $n_1 = 32$ sections for each treatment group. Tables 19, 20 and 21 are summarizing the results.

Table 19 Descriptive statistics: “area bone-porous” results for the two treatment approaches

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area bone-porous (mm$^2$)</td>
<td>32</td>
<td>0.59</td>
<td>0.07</td>
</tr>
<tr>
<td>Dynagraft only</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area bone-porous (mm$^2$)</td>
<td>32</td>
<td>0.98</td>
<td>0.12</td>
</tr>
<tr>
<td>Dynagraft + Capset</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 20 Paired T-Test: “area bone-porous” results for the two treatment approaches

<table>
<thead>
<tr>
<th>Paired parameters</th>
<th>Mean difference</th>
<th>SE</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Dynagraft only vs. Dynagraft+Capset)</td>
<td>-0.39</td>
<td>0.11</td>
<td>0.001*</td>
</tr>
<tr>
<td>Area bone-porous (mm$^2$)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SE: standard deviation.
* Statistically significant, for $p<0.05$

The mean difference is calculated by subtracting the mean of the measured parameter in the Dynagraft +Capset treatment group from the mean of the same parameter in the Dynagraft only treatment group. The negative values of the “Mean difference” indicate that the Dynagraft +Capset treatment group performed better.

Table 21 ANCOVA: “area bone-porous” results for the two treatment approaches

Estimated marginal means

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Adjusted means</th>
<th>95% Confidence interval</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower bound</td>
<td>Upper bound</td>
</tr>
<tr>
<td>Dynagraft only</td>
<td>0.705</td>
<td>0.536</td>
<td>0.874</td>
</tr>
<tr>
<td>Dynagraft + Capset</td>
<td>0.890</td>
<td>0.708</td>
<td>1.072</td>
</tr>
</tbody>
</table>

a. Evaluated at covariates appeared in the model: area defect $= 2.8940$

83
From Tables 19, 20 and 21 It can be noted that the descriptive statistics appeared to favor the combined treatment approach and paired t-test confirmed that there is a significant difference between the two treatment groups for the parameter “area bone-porous” when only submerged fixtures were considered. However, ANCOVA controlling for the covariate “area defect” failed to reveal significant differences between the two treatment groups for the parameter “area bone-porous” (overlapping of the confidence intervals boundaries indicated no significance).

Treatment assessment with light microscopy

Light microscopy assessment was performed on slides stained with toluidine blue after etching with 0.1% formic acid, with toluidine blue and light green or toluidine blue and van Gieson’s to confirm the BSEM assessment particularly in regard to correct delineation of the initial bone defect. Samples also were examined for the presence of inflammatory cells and traces of graft material, which was detected within soft connective tissue and new bone on several slides (22% of the examined sections). The identification was possible because the stains used confer it a blue color easy to differentiate from the other tissues. Also, the graft remnants had a particular configuration and were easy to identify from the surrounding tissues. (Figures 10A and 10B). No traces of Capset were identified. No differences were detected between the two treatment groups.

With the majority of the implants soft tissues were found in relation to the coronal-machined segment of the fixtures, and can occupy some reduced areas within the beads. Some sections from several implants from both treatment groups (35 out of 108) had half of their length (i.e., considering the top of the implant as reference point) in contact with soft tissue.

In implants that had not been successfully submerged we were able to describe a sulcus, (Figures 11, 12) lined by stratified keratinized epithelium that continued apically with junctional epithelium which is located either at the junction of the smooth with the porous
segment or just beyond this junction, in relation to the uppermost spherical particles of the porous surface (Figure 13). Coronally, the epithelium continues with the keratinized stratified gingival epithelium with normal architecture. Between the epithelium and the bone connective tissue rich in fibers and cells can be described. In some areas, the collagen fibers of this tissue were organized and oriented from the surface of the new bone towards the porous surface of the fixture (Figure 14). The segments of the implants that are not in contact with soft tissues are in direct contact with new or old bone (Figure 15). A minimal number of inflammatory cells were detected on majority of the samples.

For the submerged fixtures (Figures 16, 17, 18, 19) the description matches that of the non-submerged ones, with the exception that no sulcus or gap can be described, as the soft tissues, and sometimes the bone, go over the top of the fixtures. As observed with both light microscopic and BSEM examinations, new bone that formed in the Dynagraft + Capset treatment group (in 25 out of 80 backscattered SEM images from the submerged fixtures from Dynagraft + Capset treatment group) was distributed as a narrow strip or spike-like shape in a direction parallel to the implant and often to a point corresponding the top of the implant or even running over this top; The same bone distribution was observed in only 9 out of 64 examined sections from the Dynagraft only treatment group (see Figures 17 and 16, respectively).

Although the images from light microscopy may appear at first glance to be in contradiction to the statistical results, which indicate that the parameters “bone gain”, “regained bone height” and bone ingrowth fraction (for defect area) have better values for the Dynagraft + Capset treatment group, it needs to be restated that all of these measurements were done in relation to the initial bone defect depth. To better illustrate this, backscattered-SEM images from both treatment groups are presented in Figures 3A1, 3A2, 3B1 and 3B2. For example it can be seen in figures 3A1 & 3A2 (pages 68-69) that bone gain represented by the orange straight line is much greater for the combined treatment (Figure 3A2) than for Dynagraft only (Fig 3A1). The original bone defect is outlined by the red dotted line. The same is true for bone ingrowth fraction as illustrated in Figures 3B1 & 3B2 (pages 70-71).
Figure 10A. - Traces of Dynagraft Putty within soft tissues, 3 month after the treatment (arrows). Light microscopy, toluidine blue and light green stain.
Figure 10B. - Traces of Dynagraft Putty within bone, 3 month after the treatment (arrow). Light microscopy, toluidine blue and light green stain.
Figure 11. - Photomicrograph of a non-submerged implant from the Dynagraft only treatment group. Sulcus lined by stratified keratinized epithelium and continued apically with junctional epithelium (arrow). Light microscopy, toluidine blue and light green stain.
Figure 12. - Photomicrograph of a non-submerged implant from Dynagraft+Capset treatment group. Sulcus lined by stratified keratinized epithelium; Light microscopy, toluidine blue stain.
Figure 13. - Photomicrograph of a higher magnification of the junctional epithelium indicated by the arrow in Figure 11. Light microscopy, toluidine blue and light green stain.
Figure 14. - In some areas, the collagen fibers of the connective tissue were organized and oriented towards the porous surface of the fixture. Light microscopy, toluidine blue and light green stain.
Figure 15. - Direct new or old bone contact with the porous surface without soft tissue interposition (large arrows); active bone surfaces (small arrows). Light microscopy, toluidine blue and light green stain.
Figure 16. - Photomicrograph of a submerged implant from the Dynagraft only treatment group: bone runs over the implant top (arrow). Light microscopy, toluidine blue stain.
Figure 17. - Photomicrograph of a submerged implant from the Dynagraft+Capset treatment group; bone runs over the implant top (arrow). Light microscopy, toluidine blue and light green stain.
Figure 18. - Photomicrograph of a submerged implant from the Dynagraft only treatment group; soft tissues run over the implant top. Light microscopy, toluidine blue stain.
Figure 19. - Photomicrograph of a submerged implant from the Dynagraft+Capset treatment group; soft tissues run over the implant top. Light microscopy, toluidine blue and van Gieson’s stain.
VI. Discussion

In the present study it was confirmed that it is possible to induce experimental peri-implantitis with associated crestal bone loss around porous-surfaced dental implants previously placed in and integrated into dog mandible. Further, it was shown that it was possible to regenerate significant amounts of the lost bone using either a bone allograft alone or in conjunction with a barrier of calcium sulfate following the concepts of GBR (guided bone regeneration). GBR has been reported by others in animals and/or humans to be effective in the treatment of periodontal disease (Teparat et al, 1998) and of peri-implantitis (Lehmann et al, 1992; Grunder et al, 1993; Hammerle et al, 1995; Wetzel et al, 1999) around other types of dental implants i.e., threaded and rough-surfaced implants (titanium plasma-sprayed, sand-blasted and acid-etched implants). The regenerative materials used in the present study included a canine demineralized freeze-dried bone allograft in a putty consistency (Dynagraft Putty, GenSci OrthoBiologics Irvine, CA) and a resorbable calcium sulfate barrier (Capset®, Swiss NF Metal Inc., Lifecore Biomedical, Chaska, MN, U.S.A.).

The peri-implantitis model used in this study was similar to that used by others (e.g. Brandes et al, 1988; Lindhe et al, 1992). Cotton ligatures were applied and tightened around the abutments of all implant fixtures in such a way that they were forced apically, i.e. subgingivally. Animals were also withdrawn from all oral hygiene measures and fed a very soft diet to encourage plaque accumulation onto the buried ligatures (Lindhe et al, 1992; Grunder et al, 1993; Schupbach et al, 1994; Marinello et al, 1995; Ericsson et al, 1996a; Persson et al, 1999). These ligatures were replaced every 3 weeks (Lindhe et al, 1992) in order to maintain the “pocket” and further encourage the development of a mature, i.e. pathogenic, subgingival microbiota. At each ligature change standardized periapical radiographs and microbiological samples were collected again following the protocols of others (Leonhardt et al, 1992; Lindhe et al, 1992; Wetzel et al, 1999). Standardized radiographs were employed to monitor the level of crestal bone loss occurring in response to the ligature-induced disease, and hence to determine when the ligatures should be removed.
Ligatures were removed in 2 dogs after 6 weeks and in the remaining 3 dogs after 8 weeks, those being the times when at least one fixture on one mandibular side in each dog had 50% or more (i.e. 3 mm or more) of the total implant height no longer submerged in bone, as determined from radiographs. Other investigators obtained loss of 20-50% of the peri-implant bone around dental implants after 6 to 20 weeks of ligature-induced disease (Grunder et al, 1993; Jovanovic et al, 1993; Schupbach et al. 1994; Hurzeler et al, 1995; Marinello et al, 1995; Ericsson et al, 1996a; Persson et al, 1999; Wetzel et al, 1999) in dog model and with machined, titanium plasma-sprayed, titanium sandblasted and acid-etched, titanium acid-etched and HA-coated types of implants.

The pattern of bone resorption observed in the present study using radiographs, and later clinically confirmed, was mostly horizontal rather than vertical, the latter being the more preferred because of a likely better response of contained bone defects to regenerative efforts. Thus, regenerative rescue surgery of implants affected by peri-implantitis is known to be more effective in vertical-type defects (Grunder et al, 1993) as it is in the treatment of the surgically produced furcation defects affecting teeth in dogs (Pontoriero et al, 1992). A vertical bone defect is characterized by comprising one, two or three walls, which would help to contain the graft material and to support the repairing soft tissues preventing their ingrowth in the initial bone defect, especially when a barrier is used too. At the same time, in vertical bone defects the precursors of the bone cells may have shorter distances to migrate in order to reach the implant surface and start to produce new bone. In contrast, the horizontal bone defects around the implants do not have any of these mentioned advantages and this might negatively have affect the bone gain induced by our rescue procedures. The bone graft used in the present study had a putty consistency, a fact that helped with its manipulation and also provided some support for the repositioned soft tissues to maintain space for new bone formation. The poloxamer, which is the bone graft carrier that confers its putty-like characteristics, is known to be eliminated from implanted sites as soon as the second day after surgery (as discussed in detail below). This might have negatively influenced the potential desired space preservation and explain why the new bone in the Dynagraft-only group was more
evenly distributed throughout the original defects, possibly because of pressure from the
soft tissues, as observed by light microscopy and backscattered SEM. This idea might be
supported by the observation that a barrier of Capset resulted in significantly better
values linear new bone gain. Also, for some of the sections from combined treatment
group sites (as discussed in detail below) the new bone can be described as a narrow strip
or spike-like shape in a direction parallel to the implant and often to a point
 corresponding the top of the implant or even running over this top, fact that confirm the
supposition that the barrier acts to maintain the space for new bone formation.

Some investigators have suggested that the pattern of bone loss around dental implants in
response to ligature-induced peri-implantitis is influenced by the inter-implant distances
achieved at implant placement, the recommended edge-to-edge distance being 3.5 mm
(Adell et al., 1985). Grunder et al. (1993) followed this recommended inter-implant
distance in their study of treatment of ligature-induced peri-implantitis in dogs using a
GBR approach, but nevertheless reported an outcome of primarily horizontal bone loss,
probably due to overlapping and merging of defects associated with neighboring fixtures.
The mean inter-implant distance used in the present study was 4.95 mm as measured on
the radiographs taken at the time of implant placement (edge-to-edge). It seems likely
therefore that the aggressiveness of the ligature-induced disease did not permit the
formation of discrete vertical defects resulting instead in primarily horizontal bone loss.
Exceptions occurred on the mesial side of the most anterior implant and the distal side of
the most posterior implant in 10 out of 30 fixtures and elsewhere, on an unpredictable
basis where vertical defects (i.e. contained by one or more bony walls) were observed. As
well, two implants in the present study developed crater-like or saucer-shaped
circumferential infrabony defects (as assessed on radiographs), similar to those reported
by Hurzeler et al. (1995) in a study in which implants were placed with an inter-implant
distance of 7 mm. Jovanovic et al. (1993) and Machado et al. (1999) reported the same
pattern of crestal bone resorption as Hurzeler did, i.e., primarily vertical. In the above-
mentioned studies, threaded machined implants were most frequently used, but Ti
plasma-sprayed and HA-coated were employed as well. This might suggest that implant
surface geometry does not play a significant role in favoring the development of a certain
type of bone defect that results in the ligature model. Although no information is presented on the pattern of bone resorption in a study by Tillmanns et al (1998), that compared HA-coated, Ti plasma-sprayed and threaded implants in a ligature model, it was concluded that all three implant systems were equally susceptible to crestal bone loss due to ligature placement and plaque accumulation. There were no significant differences found in terms of bone loss among the systems used, with the exception of Ti plasma-sprayed surfaces, which presented significantly increased bone loss after 6 months. Correlating this information with the present findings (horizontal bone loss for porous-surfaced dental implants 6-8 weeks after ligature placement) it might be suggested that rougher surface geometries contribute to the development of more aggressive bone loss. With the exception of Hurzeler et al (1995), none of the authors mentioned the distance at which the fixtures were placed, so no correlation can be made between this parameter and the observed bone resorption patterns. Machado et al (1999) used cotton ligatures and maintained them for 1 month only, while the others (Jovanovich et al, 1993; Hurzeler et al, 1995) used silk ligatures and maintained them for 3 months. No correlations could be made to the type of the ligatures used or to the time period and the pattern of the crestal bone resorption.

Radiographs were not taken in the present study after ligature removal since the implants had been re-submerged and therefore it was no longer possible to produce standardized images. Therefore, nothing can be said about crestal bone changes after the ligatures were removed. Lindhe and Ericsson (1978) reported that bone levels in dog mandible stabilized around implants when ligatures were removed, while Marinello (1995) reported continuing bone destruction for 3 months after ligature removal, resulting in implant loss in some instances. Bone loss did appear to have been arrested in the present study after ligature removal and reinstatement of oral hygiene measures. Integration was not affected for any of the implants.

In addition to radiographs, the establishment of peri-implantitis in the present study was also verified using microbial samples analyzed by immunofluorescence microscopy to detect the presence of Porphyromonas gingivalis (Pg) at the time of the ligature
removal. Pg is a gram-negative anaerobic periopathogen known to be associated with peri-implantitis lesions in dogs and humans (Leonhardt et al, 1992). Mombelli and coworkers (1987) had suggested a criterion for established infection with Pg around dental implants in humans, namely that at least 20 Pg organisms were detected on each of 20 microscopic fields examined (in other words, at least 400 bacteria in each specimen). This criterion could not be met for any of the samples in the present study so that an alternative and qualitative method, i.e. a “present” vs. “absent” criterion, was used. As expected, it was possible to detect bacteria at the tested sites as the study progressed compared to baseline when virtually no Pg’s were present. Tillmanns et al (1998) compared the flora harbored around three different implant types, i.e. HA-coated, titanium plasma-sprayed and machined titanium alloy threaded implants placed in a dog model, and reported a similar outcome to observations in the present study, i.e. no Pg’s were detectable at baseline, when the peri-implant tissues were healthy; however, after ligatures had been present for 3 to 6 months, all tested peri-implantitis sites harbored this microorganism.

The reason that the present results differed from those reported by Mombelli et al (1987) might be that these investigators were studying human patients and collected their samples from pockets of depths 6 mm and more around implants affected by naturally-occurring peri-implantitis (as opposed to ligature-induced). In such deep pockets, a very mature subgingival plaque would be expected as such is generally the case when periodontal (or peri-implant) probing depth increases (Palmsano et al, 1991; Papaioannou et al, 1995). While pocket depths were not measured in the present study, the peri-implant pockets around the implants could not have reached such depths since no more than 4 mm of bone loss (as measured from the top of the implant fixtures) was seen.

Twelve of the 30 implant fixtures in the present study did not become re-submerged, as intended, after the rescue procedure and their healing caps were exposed partly or completely for the remainder of the study. A final microbiological assessment was done for these twelve implants immediately before the animals were terminated, and assessment of these specimens showed none to very few Pg organisms present. This was
taken to indicate that the combination of systemic antibiotics (Amoxicillin and Metronidazole) administered one week before and 2 weeks after surgery along with thorough debridement and the use of citric acid to decontaminate the implant surfaces was effective in largely eradicating the pathogenic plaque around the implants. This conclusion is further supported by the work of Mombelli and Lang (1992). These investigators used orally administrated Ornidazole, an imidazol compound with a spectrum similar to that of Metronidazole, along with surgical debridement in patients and reported after 1 year that previously compromised sites remained negative when re-tested for Prevotella intermedia, another periodontal pathogen that can be found in flora surrounding implants affected by peri-implantitis. Persson et al (1996) used an antibiotic regimen similar to the one used in the present study to treat peri-implantitis in dogs, i.e. Amoxicillin + Metronidazole for 21 days along with surgical debridement and GBR technique, and reported elimination of the inflammatory process in all peri-implant tissues, new bone formation and a small amount of re-osseointegration. Lehmann et al (1992) and Hammerle et al (1995) used the same antibiotic regimen in patients with peri-implantitis about titanium plasma-sprayed implants, and reported reduction of the probing depth and bone defect fill with bone as seen on the follow-up radiographs. None of these authors, however, reported microbiological data.

The two treatment approaches used in the present study were based on the principles of guided bone regeneration (GBR) as first described by Hurley et al (1959). Others have shown that GBR can be successful as a rescue procedure in managing peri-implantitis around other types of dental implants including threaded machined and rough-surfaced implants, i.e., titanium plasma-sprayed, sand-blasted and acid-etched implants (Lehmann et al, 1992; Grunder et al, 1993; Hammerle et al, 1995; Wetzel et al, 1999).

The goal of a “rescue” procedure for a dental implant affected by peri-implantitis is to arrest the infectious process causing bone loss and to restore osseointegration of the affected implant surfaces through promotion of new bone formation, i.e. to achieve re-osseointegration (Jovanovic et al, 1993). The first important factor in achieving this “re-osseointegration” is effective surface decontamination, i.e. removal of microbes and their
toxins. A variety of approaches have been used to achieve this necessary surface decontamination in animal models of peri-implantitis. These have included air-flow powder instruments (Gruender et al, 1993; Schupbach et al, 1994; Hurzeler et al, 1995; Machado et al, 1999), air-powder abrasive instruments in combination with citric acid (Jovanovic et al, 1993), chlorhexidine 0.12% (Wetzel et al, 1999) and delmopinol (Persson et al, 1996). In humans, clinicians have used citric acid (Deporter & Todescan, 2000), chlorhexidine 0.12% (Lehmann et al, 1992; von Arx et al, 1997), tetracycline solution 50mg/ml (Mellonig et al, 1995) or laser-activated toluidine blue (Haas et al, 2000). The efficiency of any of these agents may differ with the type of the surface involved. For example, Dennison (1994) reported that citric acid was equally effective in decontaminating machined titanium and HA-coated surfaces while the air-abrasives were equally effective for machined, HA-coated and Ti plasma-sprayed. Zablotsky et al (1992a) reported that the most effective method in terms of reducing the number of 14C-labeled E.coli endotoxin (lipopolysaccharide LPS) counts/min/sq.mm was the air-powder abrasive instrument for the metallic surfaces and citric acid for the HA-coated strips.

In the present study, citric acid (pH= 2, 1% solution) was used for 5 minutes followed by thorough saline irrigation for the decontamination of plaque-infected implant surfaces. A human case report had earlier shown this to be effective in managing peri-implantitis affecting a porous-surfaced implant (Deporter & Todescan, 2000). It was concluded that the use of citric acid had been effective in the decontamination of the porous implant surface and did not impede cell attachment and re-osseointegration.

The second important factor in achieving re-osseointegration is to use a regenerative technique promoting optimal conditions for new bone formation. A number of investigators have reported results using a variety of approaches for this purpose. For example, in a dog model Hurzeler et al (1995) compared the relative performances of freeze-dried demineralized bone matrix (FDDBM) alone, FDDBM and an e-PTFE barrier, the same barrier without any graft material, hydroxyapatite (HA) alone, HA with the barrier and debridement only in the treatment of peri-implantitis vertical bone defects around titanium implants with a machined surface. The study reported values for "bone
gain” four months after the treatment ranging from 0.5mm (SD= 0.3) for debridement alone, 1.8mm (SD= 0.3) for HA alone, 2.2 mm (SD= 0.4) for FDDBM alone, 3.6mm (SD= 0.4) for e-PTFE, 3.2mm (SD= 0.6) for e-PTFE + HA and 3.8 mm (SD= 0.8mm) for FDDBM +e-PTFE, as measured from the bottom of the defect to the most coronal point of the new bone. When the results from GBR techniques were compared, no statistically significant differences were found. The same was true when the performances of graft materials were considered.

Machado et al (1999), in an similar study compared a GBR technique with e-PTFE, the use of a mineralized bone xenograft alone (Bio-Oss, Osteohealth Co., NY, USA) and the combination of the two for the treatment of peri-implant vertical bone defects created by the use of cotton ligatures around commercially pure titanium implants in dogs. The authors observed that bone graft alone induced bone gain of 1.58mm (SD= 0.62), bone graft in a GBR technique induced 1.6 mm (SD= 1.12) bone height gain, Debridement + GBR (PTFE membrane alone) 1.37mm (SD= 0.83) and control (debridement only) had 0.85 mm bone height gain (SD= 0.41). These results confirmed those reported by Hurzeler et al (1995), that bone graft alone or in combination with a membrane was most effective in terms of new bone formation. The much smaller linear bone gains reported by Machado et al (1999) when compared with Hurzeler’s results might be the consequence that Machado et al (1999) evaluated the results of their treatment after only 1 month.

Wetzel et al (1999) tested the efficacy of debridement only vs. GBR using a barrier of e-PTFE only to manage peri-implantitis defects in beagle dogs associated with implants of various surface finishes, i.e. machined titanium implant vs. TPS vs. titanium sand-blasted and acid-etched in a vertical type bone defect. They reported a mean gain in bone height of 2.36mm for the GBR treatment compared to only 0.52 mm for debridement alone. The greatest gain was 2.6 mm (SD= 0.69) and with the TPS implants. Interestingly, the authors reported statistically significant (p<0.05) greater bone fill with GTR when compared with debridement alone, regardless of the surface geometry of the tested implants.
Grunder et al (1993) attempted to treat horizontal bone defects around pure titanium implants using a barrier of e-PTFE in the dog model. They reported no gain in bone height (-0.1 mm) after the treatment and speculated that this poor result was related to the horizontal configuration of the defects, and to the fact that the membranes became exposed 1 week after treatment with associated bacterial contamination. Indeed, e-PTE membranes are known to be highly susceptible to this complication (Wetzel et al, 1999; Haas et al, 2000) and this is one reason that calcium sulfate was used as the GBR barrier material in the present study.

In humans, the use of GBR techniques has been reported to be successful in the treatment of peri-implantitis. For example, Lehmann et al (1992) reported a mean bone gain of up to 5 mm (re-osseointegration of 55%) after GBR using e-PTFE membrane. Hammerle et al (1995) using the same implant type, titanium plasma sprayed and the same antibiotic regimen (Amoxicillin and an imidazolic compound) and treatment approach (GBR using e-PTFE membrane) as Lehman and his colleagues did, reported 40% re-osseointegration and mean bone gain of 2.3 mm. In both studies the bone defect had a vertical pattern and the re-evaluation was done 6 months post-treatment using radiographs.

In the present study, two regenerative surgical treatment approaches were compared, one with an osteoinductive allograft material alone and one with this same material subsequently covered with a barrier of calcium sulfate. A control group in which no materials were used was not included because of limited resources and since such a procedure is known from clinical experience not to be successful in managing peri-implantitis around porous-surfaced implants. The osteoinductive allograft material used was prepared on special request by GenSci OrthoBiologics (Irvine, CA) and is referred to by the trade name, Dynagaft Putty®. It was partially prepared as originally described by Urist (1965) using a series of steps including demineralization with hydrochloric acid in order to expose bone morphogenetic proteins (BMP’s), glycoproteins known to encourage bone cell differentiation and bone formation. While a commercially-available version of this material contains human demineralized, freeze-dried bone allograft
particles, for the present study the material was made containing canine demineralized, freeze-dried bone particles because of known species specificity of BMP's and associated negative effects of untoward immune responses on osteoinduction (Bessho et al, 1992). The bone allograft was prepared in a synthetic resorbable aqueous carrier (a poloxamer polymer that stiffens as the temperature rises, i.e. a “reverse phase polymer”) in order to confer certain desirable handling characteristics including easy manipulation, good graft stability, possible lack of need for a barrier to contain the graft in situ, and full resorbability while still preserving space for a sufficient time interval to allow for new bone formation. The graft material was tested by the manufacturer for its osteoinductive activity both before shipping and again after its use in surgery with remaining portions and found to be biologically active based on alkaline phosphatase activity (control group had 11 alkaline phosphates units nmol/ml/h, while our graft exhibited 69+/− 14 alkaline phosphatase units nmol/ml/h).

Clokie et al (2000) and Coulson et al (1999) published results of experiments in animals and reported osteoinductive properties with great potential for clinical use in cranial defects and also in bone defects associated with dental implants.

The barrier material chosen for use in this study was medical grade calcium sulfate ("plaster of Paris") available commercially as a product called Capset®. It was chosen because of its favorable performance as a substrate for gingival fibroblasts in vitro (Payne et al, 1996) and because it has been reported to function effectively as a barrier in the regenerative treatment of periodontally-compromised teeth with no apparent post-operative complications (Sottosanti et al, 1993; Anson, 1996; Kim et al, 1998). As already indicated, this material was preferred over a non-resorbable barrier like e-PTFE because it is not subject to the same complications and, being resorbable, eliminates the need for a second surgical intervention for the removal of the barrier. A possible added advantage is that its calcium content may accelerate the rate of mineralization of the new bone (Peltier & Orn, 1958; Yamazaki et al, 1988).
The likely healing sequence with the Dynagraft material starts with the elimination of the resorbable carrier by the second day after implantation as reported by the manufacturer and demonstrated experimentally (Schmolka et al, 1972; Li et al, 1996). Following this, the likely sequence of events is one resembling that when demineralized bone matrix alone is used as graft as described by Wang et al (1999) from day 2 to day 28 after implantation in the rat cranial defect model. The healing sequence is as follows: at day 2 the authors observed small capillaries and large spindle-shaped cells with minimal matrix formation between the grafted bone particles along with a fibrin clot, extravasated erythrocytes and a very small number of polymorphonuclear leukocytes surrounding the particles of FDDBM. By day 3, alkaline phosphatase-positive cells with morphological characteristics of osteoblasts were detected. Bone matrix in small amounts and osteocytes embedded in it can be observed by day 4. Some of the matrix can be mineralized by day 5. By day 6 to 10, synthesis of woven bone lined with osteocytes continued. At day 6-7 very few cartilage cells were seen. The formation of bone marrow was evident 3 weeks after implantation. By 28 days, the cranial rat defect was healed. (Wang et al, 1999). In the present study, elevated local calcium levels derived from the Capset may have accelerated this process by increasing the rate of mineralization of the new bone (Peltier & Orn, 1958; Yamazaki et al, 1988).

The resorbability of demineralized, freeze-dried bone particles and of other bone fillers currently used has been reported to be variable. For example, Buser et al (1998) evaluated the performance of FDDBM allograft compared to collagen sponge, tricalcium phosphate and coral-derived HA using blood clot and autograft as control, in the treatment of peri-implant defects in miniature pigs. Using light microscopy assessment, these investigators were still able to identify remnants of FDDBM graft material within the newly formed bone after 6 months of remodeling (14.2 % volume). HA treated specimens presented 30% volume remnants of the filling material while the non-viable autogenous bone chips occupied 11% volume after 6 months from the treatment. The tricalcium phosphate was also detected 6 months after the treatment and represented 7.5% by volume.
Simion et al (1994) compared in humans the ability of e-PTFE alone or used along with autogenous bone chips, human FDDBM, Grafton, a new form of demineralized allograft bone tissue processed gel, to enhance bone regeneration around dental implants placed into fresh extraction sockets. Using a specially designed harvester to access the implant cover screws while allowing for bone biopsy collection without disturbing the implants, the authors reported that after 6 months no signs of resorption of the FDDBM and Grafton particles were seen, although they were partially remineralized, as detected using von Kossa staining technique, while the autogenous bone chips were completely integrated with the newly formed bone. Hammerle et al (1998) used deproteinized bovine bone mineral to treat peri-implant bony defects in monkeys, and was able to identify graft particles embedded in bone and in the connective tissue 6 months following treatment. Hurzeler et al (1995) was able to identify HA that was used as a graft material after 3 month from the treatment.

In the present study, light microscopic assessment at the end of the 3-month healing interval following grafting detected only very small amounts of the graft material within the soft tissues and newly formed bone. Two to 6 histological preparations from each fixture were examined for graft remnants and the graft could be seen on 22% of the slides of both treatment groups. No retained calcium sulfate barrier material was identifiable in any of the samples.

It was the intent following rescue surgery to re-submerge all implants during the 3-month healing interval because a closed environment would reduce the risk of bacterial contamination following GBR. However, with twelve of the thirty treated implants this was not possible so that the healing caps of these twelve implants remained partially or completely exposed until the end of the experiment. Therefore, it was possible to examine for differences in the healing response around submerged vs. non-submerged implants. The main difference between the two situations was the formation of a gingival sulcus in association with the non-submerged implants. Coronally, the epithelium of the sulcus presented as a keratinized stratified gingival epithelium with normal architecture, and similar to that reported by other investigators using dental implants with
commercially pure titanium implants with acid-etched surfaces in dogs (Grunder et al, 1993; Schupbach et al, 1994). The sulcus was delineated apically by a junctional epithelium, which extended most frequently to the junction between the machined and porous-surfaced segments of the implant fixture, or just beyond this junction, i.e. next to the uppermost spherical particles of the porous surface. In both treatment groups in 66% of the examined samples the implants have their most coronal third of the collar and porous surface in contact with soft connective tissues. Also the connective tissue occupied some small areas within the porous surface. Of the examined sections on 32% the implants had up to half of their length in contact with soft tissue. The segments of the implants that were not in contact with soft tissues were in direct contact with new or old bone. Between the base of the sulcus and the underlying bone connective tissue was seen. On the assessed samples, the connective tissue appeared rich in fibers and cells. There was a tendency for the collagen fibers of this tissue to be somehow organized and oriented from the surface of the new bone towards the porous surface of the fixture. The magnification used for the light microscopy assessment, i.e., 10X, did not allow us to characterize the relationship of the collagen fibers with the porous surface. Fibroblasts could be seen on all samples within the connective tissues.

The submerged samples had a similar histological appearance to the non-submerged ones in terms of the type of tissues observed, their architecture and position in relation with the implant surface with the exception that no sulcus was observed, the submerged fixtures healing caps being covered by soft tissues and, sometimes, by bone.

On all examined sections a significant length of the porous surface i.e., the one that was not in contact with the soft tissues, was embedded in new or old bone with normal architecture. This bone was in direct contact with the spherical particles of the porous surface with no interposition of soft tissues as earlier described following initial integration of porous-surfaced implants in dog mandibles (Deporter et al, 1986). Minimal number of inflammatory cells was identified in the majority of samples.
On all samples variable areas of the new bone formed in the initial bone defect were observed, these observation being in accord with those of other investigators that have used grafting techniques to treat the peri-implant bone defects (Hurzeler et al, 1995; Wetzel et al, 1999).

The results of the present study indicated a mean linear “bone gain”, as measured from the bottom of the original defect to the most coronal point of new bone in contact with the porous surface, of 0.60 mm (SD= 0.583) for the Dynagraft-only group and 0.89 mm (SD= 0.881) for the Dynagraft + Capset group. This represented a mean 32% re-osseointegration for the Dynagraft-only sites and a mean 48% re-osseointegration for the Dynagraft + Capset sites. The mean defect depth as measured on backscattered SEM images from the smooth-porous junction to the bottom of the defect was 1.99 mm. The difference between the two treatments was statistically significant, p<0.05, as demonstrated by paired t-test. A possible explanation for this may be that the Capset had, as intended, a protective role delaying the ingrowth of gingival fibroblasts and epithelial cells into the grafted defects thus preserving more space for new bone formation. As observed with both light microscopic and BSEM examinations, new bone that formed in the Dynagraft + Capset treatment group (in 25 out of 80 backscattered SEM images from the submerged fixtures from Dynagraft+Capset treatment group) was distributed as a narrow strip or spike-like shape in a direction parallel to the implant and often to a point corresponding the top of the implant or even running over this top. This architecture may have been the result of the Capset “squeezing” some putty upwards against the implant surface during flap closure and suturing, and then holding it in place long enough for it to become organized on its way to new bone formation. New bone in the Dynagraft-only group was more evenly distributed throughout the original defects and to a lesser height in relation to the top of the implant, although in 9 out of 64 of the submerged implants in this treatment group, the new bone was distributed as described above for the combined treatment, i.e., a narrow strip or spike-like shape in a direction parallel to the implant and often to a point corresponding the top of the implant or even running over it. A comparison with the results reported by other investigators following treatment of experimentally-induced peri-implantitis in the dog model using GBR techniques can be
made in terms of reported “bone gain” height as a linear measurement from the bottom of the defect to the most coronal point of the new bone formed and in terms of re-osseointegration (how much of the defect was filled with bone).

Grunder et al (1993) used GBR with an e-PTFE barrier, in a horizontal defect and after 3 month reported barrier exposure and no bone gain (-0.1mm). Because the overall pattern of the bone resorption in the present study was also horizontal, Grunder’s result (1993) study is the only one to which a direct comparison can be made. In contrast to Grunder et al (1993) findings, in the present study mean linear “bone gain”, as measured from the bottom of the original defect to the most coronal point of new bone in contact with the porous surface, were determined to be 0.60 mm (SD= 0.58) for the Dynagraft-only group and 0.89 mm (SD= 0.88) for the Dynagraft + Capset group. This represented a mean 32% (SE= 29%) re-osseointegration for the Dynagraft-only sites and a mean 48% (SE= 0.39%) re-osseointegration for the Dynagraft + Capset sites.

The results from other animal studies on which vertical bone resorption pattern was observed and treated by different approaches are presented below. Because a vertical pattern of initial bone defect was reported in these studies, a direct comparison with the result of the present study was not possible. Hurzeler et al (1995) reported 3.8 mm (SD= 0.8) bone gained (90% re-osseointegration) 4 months post treatment using FDDB+ GBR technique, in vertical defects. Machado et al (1999) reported 1.58mm (SD= 1.12) for the same technique, 1 month after treatment again with vertical of bone defects. The initial depth of the bone defect is not reported by Machado, so that one cannot calculate the percent re-osseointegration. The differences between the values reported by the two studies might be due to the fact that Machado et al (1999) used a xenograft material and their evaluation was done after 1 month. Wetzel et al (1999) reported 2.6 mm (SD= 0.69), 72.6% re-osseointegration with the TPS implants, 6 month after treatment of vertical bone defects using GTR technique. Persson et al (1996), assessed the results 4 months after the treatment (GBR using e-PTFE only) and reported, bone gain 0.32 mm, (17% re-osseointegration), in a vertical defect.
In the present study, in addition to the “bone gain” and regained bone height (RBH), several other objective parameters of bone regeneration were studied including the area of new bone, the absolute contact length of bone in contact with the outermost layer of particles of the porous surface originally affected by the defect (ACL-d), the contact length fraction for this interface (CLF-d) and the bone ingrowth fraction (BIF-d) as well. The area of new bone measurements (done taking the top of the fixtures as fixed reference point with the two areas of the new bone i.e., related to each machined and porous surfaces, considered collectively) indicated a better overall outcome in the Dynagraft + Capset treatment group (1.09mm$^2$, SE= 0.069) when compared to Dynagraft-only group (0.98mm$^2$, SE= 0.072). This difference was, however, not statistically significant when tested by paired t-test (p= 0.283). Likewise, when ANCOVA was used to control for the covariate “area defect” (i.e. for variations in the area of the original defects being grafted) no significant difference for the parameter “area bone” was found between the two treatments. Use of the paired t-test indicated a significant difference (p<0.05) between the two treatment approaches for the parameter BIF-d, the difference again favoring the Dynagraft + Capset treatment group and again a possible explanation for the difference may be the protective effect affording by the Capset which excluded non-bone cells, and allowed for undisturbed ingrowth of bone cells into the porous- surface. Differences in ACL-d and CLF-d between the two treatment approaches were not statistically significant (p= 0.401 and respectively p= 0.156).

When the paired design was used to test differences between the two treatment groups for the submerged implants only, again the RBH and “bone gain” were significantly better (p<0.05) for Dynagraft + Capset treatment group; also “area bone” had significantly better values (p<0.05) for Dynagraft + Capset treatment group when only the submerged fixtures were considered. This might be interpreted as a possible advantage of submerging the implants after treatment, offering them an isolated and more protective environment.
Further, ANCOVA was used to compare the performance of the two different rescue procedures only for the submerged fixtures but the analysis failed to detect statistically significant difference for parameter “area bone” controlling for the covariate “area defect” between the two treatment approaches when only the submerged fixtures were considered.

For the parameter “area bone-porous” which was calculated separately considering the area of new bone that formed in relation to the porous surface only and ignoring the area of new bone that formed in relation to the machined collar, in both situation (when testing sections from all implants in the study and when only the sections from the submerged fixtures were considered), descriptive statistics results appeared to favor the combined treatment approach. However, paired t-test failed to reveal significant differences between the two treatment groups for the parameter “area bone-porous” when sections from all implants (i.e., submerged and non-submerged fixtures) were considered and the same test confirmed that there is a significant difference (p<0.05) between the two treatment groups for the parameter “area bone-porous” when only submerged fixtures were considered. These observations suggest the possible protective effect provided by the secluded environment allowing for increased amount of new bone formation. However, ANCOVA controlling for the covariate “area defect” failed to reveal significant differences between the two treatment groups for the parameter “area bone-porous” in both situations.

The parameters absolute contact length, contact length fraction and bone ingrowth fraction were also determined for the non-defect implant surface, i.e. the segment of porous surface that had not been affected by the peri-implantitis (ACL-nd, CLF-nd and BIF-nd). These data were compared to corresponding data for the segments of porous surface localized to the defect area (ACL-d, CLF-d and BIF-d) using the paired t-tests. These comparisons revealed statistically significant differences (p= 0.00) between ACL-d and ACL-nd, CLF-d and CLF-nd, and between BIF-d and BIF-nd, the bone contact and the bone ingrowth being greater in relation to the non-defect segment of porous implant
surface. There could be several explanations for this difference. Firstly, the two segments had been in contact with bone-forming cells for different periods of time (for non-defect segments since the original osseointegration process, i.e. for a total of 8 months; for defect-associated segments for only 3 months, i.e. since rescue). Secondly, it is possible that the acid-decontaminated and previously plaque-infected porous implant surface associated with the defects was not as hospitable for bone-forming cells as the original sterile implant surface, a reasonable assumption.

Limitations of the present animal model system and experimental design should also be considered when evaluating the results of the present study. All surgical procedures including the follow-up radiographs were performed under general anesthesia, requiring the assistance of professionals and close supervision of the animals after procedures. Teeth extraction was fairly difficult because of both, bone rigidity and specific anatomy of teeth. Once the cotton ligatures were placed and animals were fed soft diet, gross plaque accumulated fairly rapid. As demonstrated later by the follow-up radiographs the bone defects induced had a horizontal pattern possible either because the implants were placed too close together (4.95 mm), fact that facilitated overlapping of the defects from neighboring implants, or because of the impossibility to quantify and control the aggressiveness of the ligature-induced condition. The use of thinner ligatures, as 4-0 silk ones, might facilitate the development of more confined bone defects. The pattern of bone resorption observed in the present study might affect the treatment outcome. Absence of a control treatment group should also be mentioned as a limitation of the present study. Possible controls that could have been used include: no treatment after ligatures removal, orally antibiotics with no local procedures, systemic antibiotics along with local debridement, orally antibiotics along with local debridement and a resorbable membrane in a guided regeneration procedure or orally antibiotics along with local debridement and a resorbable membrane and the use of a bone graft material in a guided bone regeneration procedure. The present study had an original approach because it was the first animal experimental study when peri-implantitis was induced, using the ligature model, and treated around porous-surfaced dental implants. However, this is a pilot study and needs further research effort for complete validation.
From all the above, it can be concluded that the both treatment procedures were relatively successful, especially since the defects being managed were primarily horizontal. No fixtures were lost before or after the treatment and significant portions of the porous surface previously affected by peri-implantitis became re-osseointegrated. There would, however, appear to be some advantage to using the combined approach because as demonstrated by the parameters that characterize re-osseointegration, Capset had a positive effect allowing for greater new bone formation.
VII. Conclusions

1) An experimental model for peri-implantitis around porous-surfaced dental implants using the ligature approach was established: in 6-8 weeks following ligature placement, hygiene withdrawal and soft diet administration, gross plaque accumulation occurred; radiographic exams revealed crestal bone loss of up to 3 mm (more than 50% of the total implant length) from the original bone level and microbiological assessment indicated the presence of *Porphyromonas gingivalis* at each peri-implantitis site.

2) Assessment of prepared sections using BSEM and statistical treatment of the data indicated that both treatment approaches i.e., Dynagraft +Capset and Dynagraft only, were effective in terms of inducing new bone formation and allowing for re-ossseointegration of previously disease-affected porous implant surface.

3) The combination treatment (i.e. Dynagraft +Capset) group performed better in terms of regenerated bone height and increased bone ingrowth fraction, possibly as the result of the protective effect of the Capset, which may have prevented soft tissue invasion of the surface of the Dynagraft material.
Appendix

Toluidine blue: Solution 1: 0.3% toluidine blue
   Solution 2: 2% sodium borate

Mix solutions 1 and 2 (1:1 ratio)
Heat to 50°C
Stain for 15 minutes
Rinse with 100% ethanol

Van Gieson: Acid fuchsin 1% aqueous 10-15ml
   Saturated picric acid 100ml
Stain 5 minutes at room temperature

Light green: light green 0.5g
   Distilled water 98ml
   Acetic acid 2ml
Stain 30-60 seconds at room temperature

Results
Toluidine blue and light green:
Nuclei- light blue
Cytoplasm- dark blue
Osteoid seam- dark blue
Bone matrix- green
Soft tissue elements- dark blue

Van Gieson
Nuclei- dark blue
Cytoplasm- light blue
Osteoid seam- blue green
Bone matrix- yellow orange to autumn orange
Soft tissue elements- blue to blue green
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