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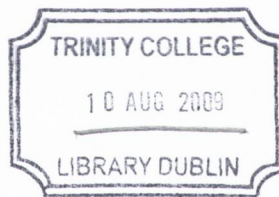
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Effect of Surface Contamination on Re- osseointegration of dental implants surrounded by circumferential bone defects

A thesis submitted in partial fulfilment of Doctorate in Dental Surgery
(D. Ch. Dent.) Periodontology

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2008



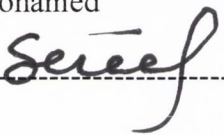
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Seif Mohamed

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DEDICATION

To my wife, Nuha for her patience and continuous support over the last three years.

To my children, Nasr, Nafissa and Mugtaba.

To the memory of my parents who were wishing to see this moment.

Acknowledgements

To Professor Noel Claffey, for his trust, encouragement, guidance and continuous support.

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To peter O'Reilly for his help.

SUMMARY

AIM OF THE STUDY: This study was designed to evaluate the effect of surface contamination on re-osseointegration of dental implants surrounded by circumferential bone defect and to compare osseointegration around Osseotite[®] implants (Biomet 3I, Palm Beach Gardens, USA) with that around Nanotite[™] implants (Biomet 3I, Palm Beach Gardens, USA) in beagle dogs.

MATERIALS AND METHODS: Four adult beagle dogs and 16 dental implants, eight commercially pure titanium 3.25mm x 13mm Osseotite[®] implants (Biomet 3I, Palm Beach Gardens, USA) and eight commercially pure titanium 3.25 x 13mm Nanotite[™] implants (Biomet 3I, Palm Beach Gardens, USA) were used. The lower premolars (P1, P2, P3 and P4) were extracted. Following 3 months of healing, buccal and lingual mucoperiosteal flaps were raised and 4 implants (Two Osseotite[®] and two Nanotite[™]) were partially inserted in the left side of the mandible in each dog.

Following five weeks of healing period, the implants were removed from the left sides, decontaminated by tooth brush and saline and placed into freshly prepared sites to the full implant length on the right side of each mandible. The coronal 5 mm of each implant was surrounded by 1.0 mm circumferential bone defect. Following 12 weeks of healing period, the dogs were sacrificed and the mandibles on experimental sides were harvested.

Hard tissue ground sections were prepared of biopsies taken at the end of the study and histometric measurements were obtained.

RESULTS: The mean percentage of bone in direct contact with the implant surface (BIC) was 41%, 81% and 83% for the part that was previously contaminated and surrounded by bone (part 1), the part that was previously opposed by soft tissue alone

(part 2) and the part that encased by bone (part 3) respectively. The mean percentage of BIC for part 1 was significantly lower than part 2 and part 3 ($p = 0.0001$). The mean percentage of the bone area within threads for part 1, part 2 and part 3 was 55%, 80% and 79% respectively. Part 1 showed significantly lower percentage of bone area within threads than part 2 and part 3 ($p = 0.0007$). The mean percentage of BIC for Osseotite[®] implant (type 1) and Nanotite[™] implant (type 2) was 72% and 65% respectively. The difference was not statistically significant ($p = 0.23$). The mean percentage of bone area within threads for type 1 and type 2 was 65% and 78% respectively. The mean percentage of bone area within threads was found to be significantly higher for type 2 implant than type 1 implant ($p = 0.0299$).

Conclusions: The result demonstrated that osseointegration can occur to the implants surfaces that were previously contaminated and surrounded by bone defects. Nanotite[®] implants showed significantly greater bone area within threads than Osseotite[™] implants.

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CHAPTER 1

INTRODUCTION

The use of osseointegrated implants in the rehabilitation of partial or complete edentulism is becoming a common treatment procedure. For the last 30 years, favourable long-term results of implant-supported and implant-retained prostheses have been reported in complete and partially edentulous patients. (Adell *et al.* 1981, Albrektsson *et al.* 1988).

Occasionally however, implant threads become exposed as a result of bacterial-induced peri-implant destruction. This destruction has been termed peri-implantitis and the objective of therapy for this condition is to regain integration of the implant with bone.

Various treatment modalities have been used to treat peri-implantitis in humans and animal models including debridement & decontamination of the exposed implant surface, access flap procedures, use of adjunctive anti-microbial agents and regenerative procedures with membranes/grafts. (Singh *et al.* 1993, Gründer *et al.* 1993, Jovanovic *et al.* 1993, Persson *et al.* 1996, Wetzell *et al.* 1999) Although all of these treatment modalities were found to be effective in cleaning titanium surfaces and to allow for soft tissue healing and bone re-fill in the defects, only limited re-osseointegration occurred.

Failure of re-osseointegration following induced peri-implantitis has been attributed to many factors including contamination of implant surface, bony defects surrounding the implants and other factors such as clot adhesion/stability and cellular migration/differentiation.

Re-osseointegration to surfaces that were plaque contaminated was considered to be either extremely difficult or impossible to obtain. (Gründer *et al.* 1993, Ericsson *et al.* 1996, Persson *et al.* 1996, 1999, 2001, Wetzel *et al.* 1999).

In an experiment in Labrador dogs, peri-implantitis lesions were treated by systemic antimicrobial therapy and local measures including flap elevation, curettage and chemical decontamination of the exposed implant surface (Ericsson *et al.* 1996). The abutment parts were removed, cleaned in amino-alcohol (1% delmopinol HCL) and autoclaved, while the fixture components were cleaned thoroughly with the same amino-alcohol. It was observed that this regime when accompanied by a careful plaque control program during healing resulted in the elimination of the inflammatory lesions, but re-osseointegration failed to occur to titanium surfaces previously exposed to plaque (Ericsson *et al.* 1996). A similar result was obtained when the contaminated abutments were discarded, and pristine cover screws were placed over the cleaned fixtures, which were subsequently submerged (Persson *et al.* 1996).

In an attempt to avoid chemical manipulation of the fixture surface, Persson *et al.* (1999) restricted the cleaning of the exposed implant to mechanical means using a rotating brush and an abrasive (pumice). This treatment resulted in marked bone formation in the crater like defects and the establishment of a connective tissue capsule that separated the newly formed bone from most of the cleaned implant surface. The authors concluded that neither chemical nor mechanical treatment of the contaminated implant surface established conditions conducive for re-osseointegration.

Persson *et al.* (2001a) used implants comprised of two parts, one apical and one coronal part that were joined with a connector. Such fixtures were first installed in the edentulous premolar region of Labrador dogs and peri-implantitis was subsequently induced. During treatment of the inflammatory condition (systemic antibiotics and surgical granulation tissue removal), the upper part of the fixture was removed and substituted with identical but pristine part. Healing resulted not only in resolution of inflammation but also in re-osseointegration of the pristine fixture. The author concluded that “the quality of the titanium surface is of decisive importance for both osseointegration and re-osseointegration.”

Kolonidis *et al.* (2003) investigated the direct influence of surface contamination on re-osseointegration of smooth surfaced dental implants in Labrador dogs. Results demonstrated that osseointegration can occur on surfaces that were plaque contaminated and cleaned by different methods. The findings of Kolonidis *et al.* (2003) were consistent with Alhag *et al.* (2008) who evaluated the effect of surface contamination on re-osseointegration of rough surfaced dental implants in beagle dogs.

It has also been demonstrated in animal models that osseointegration can occur when sterile implant surfaces are surrounded by bony defects. (Scipioni *et al.* 1997, Paolantonio *et al.* 2001, Botticelli *et al.* 2003a, Polyzois *et al.* 2007, Abushahba *et al.* 2008).

According to these results neither the implant surface contamination nor the bony defect surrounding the implant seems to prevent re-osseointegration.

Titanium dental implants have either machined (turned) or rough (textured) surfaces. Recently machined surfaced implants have been superseded by the rough surfaced implants. Many different techniques have been used to modify dental implants surfaces such as titanium plasma spray, grit-blasting, acid-etching, hydroxyapatite coating (HA) and anodization. One of the most recent surface modifications is the Discrete Crystalline Deposition (DCD) process as used in the Nanotite™ implants. This process involved deposition of nano-structured calcium phosphate on the surface of the dual acid-etched implant resulting in discrete crystal deposits of 20-100 nanometers in length. The DCD process increases the micro-surface area by 200%. There is lack of studies regarding osseointegration of the newly introduced Nanotite™ surface implants in comparison to the other implant surfaces such as Osseotite® surface implants.

The aim of this study was to investigate the effect of the surface contamination on re-osseointegration of dental implants surrounded by circumferential bone defects and to compare the amount of osseointegration around Osseotite® implants (Biomet 3I, Palm Beach Gardens, USA) with that around Nanotite™ implants (Biomet 3I, Palm Beach Gardens, USA) in beagle dogs.

CHAPTER 2

LITERATURE REVIEW

2.1 Osseointegration

Brånemark *et al.* (1969) performed animal experiments and indicated that it was possible to establish a direct bone anchorage to an implanted metal device provided that a number of guidelines were followed. This was documented in the first clinical report published some years later (Brånemark *et al.* 1977).

Brånemark *et al.* (1977) described this relationship between the implant surface and bone for which they coined the term osseointegration. This has been described as “a direct functional and structural connection between living bone and the surface of a load carrying implant with persistent stability where there is no progressive movement”.

In the 1970s there were no methods available to section intact bone to metal specimens and hence the histologic evidence of osseointegration remained indirect. Newly developed techniques were used to cut through uncalcified bone and implant without previous separation of anchorage and since then direct bone to implant contact was proved beyond doubt (Schroeder *et al* 1981).

Under light microscopy osseointegration can be defined as the direct attachment of bone to an implant surface without an interposed soft tissue layer (Albrektsson *et al.* 1982 & 1983). However, at the electron microscopic level, bone has been shown to be approximately 20 nm from the implant surface, or in contact with the implant surface (Albrektsson *et al.* 1985, Listgarten *et al.* 1991).

Zarb & Albrektsson (1991) redefined osseointegration as “a process where by clinically asymptomatic rigid fixation of alloplastic materials is achieved and maintained in bone during functional loading”. This was based on stability instead of histological criteria, as the exact degree of bone attachment was difficult to identify.

Albrektsson *et al.* (1981) presented a series of factors that needed to be controlled in order for reliable osseointegration to ensue. These factors were refined by Adell *et al* (1985) and eventually summarized by Albrektsson & Sennerby (1990) and they included the following:

1. Biocompatibility and inertness of implants.
2. Implants design,
3. Surface characteristics,
4. Host bone quality and quantity,
5. A traumatic surgical technique ,
6. Loading of implant after sufficient healing periods.

Many factors that have been suggested to jeopardize or prevent Osseointegration include:

1. Improper preparation of the recipient site which results in undue hard tissue damage such as bone necrosis.
2. Bacterial contamination and extensive inflammation of the wound.
3. Inadequate mechanical stability of an implant following its insertion,
4. Premature loading of implant,
5. Irradiation,
6. Smoking.

Bain and Moy (1993) reviewed 2,194 Brånemark implants over a 6 year period. They found that the percentage of failure in smokers was 11.2% compared to 4.7% for non-smokers. A retrospective study by De Bruyn & Collaert (1994) reported that failure before loading is 9% in smokers compared to 1% in non-smokers. Recent systematic reviews documented that smoking interferes with the prognosis of dental implants and associated with complications such as peri-implantitis (Strietzel *et al.* 2007, Lindhe *et al.* 2008).

2.2 Implant surface

Titanium dental implants have either machined (turned) or rough (textured) surfaces. Smooth surfaced implants have been used successfully for more than 30 years in treatment of partially dentate and fully edentulous patients (Adell *et al.* 1981, Albrektsson & Sennerby 1990). Recently machined surfaced implants have been superseded by the rough surfaced implants. Many different techniques that have been used to modify dental implants surfaces such as titanium plasma spray, grit-blasting, acid-etching, hydroxyapatite coating (HA) and anodization. Descriptive histological or histomorphometric experiments in humans & animal models have shown that rough surfaced implants have a greater bone-implant contact area than do implants with smoother surfaces (Buser *et al.* 1991, Godfredsen *et al.* 1992, Cochran *et al.* 1998, Wennerberg *et al.* 1998, Ivanoff *et al.* 2001).

Buser *et al.* (1991) compared 6 different implant surfaces with regard to bone-implant contact area. The implants were placed in the metaphyses of the tibia and femur of miniature pigs. After a healing period of 3-6 weeks histomorphometric analysis had

shown that machined and medium blasted implants demonstrated the lowest percentage of bone-implant contact (mean from 20-25%). Large sandblasted and TPS implant surfaces had a mean of 30-40% bone-implant contact area, and large sandblasted and acid-etched (SLA) had a mean of 50-60% of bone-implant contact area. This result indicated that the extent of bone-implant interface was positively correlated with an increasing implant surface roughness.

Ivanoff *et al.* (2001) in a study on human volunteers compared the smooth surfaced implants with sandblasted rough surfaced implants regarding bone-implant contact area. Micro-sized type implants were placed in maxilla & mandible and allowed to heal for 6 months and 4 months respectively. Histological examination revealed that rough surfaced implants demonstrated significantly larger amount of bone-implant contact area than the smooth surfaced implants.

In another human experiment Lazzara *et al.* (1999) compared thermal dual acid-etched Osseotite® implants (Biomet 3I, Palm Beach Gardens, USA) with machined implant surface regarding bone-implant contact. After a healing period of 6 months, results showed that rough surfaced implants demonstrated twice the amount of bone-implant contact area compared to machine surfaced.

Biomechanical studies assessing removal torque values have shown that rough surfaced implants require higher forces to be removed from the bone than do implants with smooth surface (Wilke *et al.* 1992, Carr *et al.* 2001).

Carr *et al.* (2001) compared torque failure levels of commercially pure titanium, titanium alloy and HA-coated implants in baboons. After a healing period of 3-4

months results showed that HA-coated implants demonstrated significantly greater torque removal values when compared to smooth surfaced implants.

One of the most recent surface modifications is the Discrete Crystalline Deposition (DCD) process. This process involved deposition of nano-structured calcium phosphate on the surface of the dual acid-etched implant resulting in discrete crystal deposits of 20-100 nanometers in length. The DCD process increases the micro-surface area by 200%*

Orsini *et al.* (2007), in a randomized controlled double-blind study, evaluated implants with Nanometer-scale calcium phosphate added to the dual acid-etched surface in the human posterior maxilla. One 2 x 10 mm site evaluation implant (SEI) with nanometer calcium phosphate added to the dual acid-etched surface (test) and one SEI dual acid-etched surface without treatment (control) were placed in posterior maxilla of 15 patients. After 2 months of healing, histologic and histomorphometric analysis revealed that implants with the treated surface (test) demonstrated significantly greater bone-implant contact area than the control. The authors concluded that the nanometric deposition of calcium phosphate crystal to the dual acid-etched surface implants can be clinically advantageous for shortening the implant healing period, providing earlier fixation and minimizing micromotion, thus allowing earlier loading and restoration of function for implants placed in areas with low-density of bone..

The possible mechanisms by which rough surfaced implants produce greater bone-implant contact area than smooth surfaced include:

- 1- Rough surface increases the surface area for bone-implant contact.

2- Retention of fibrin clot at the interface zone and osteoconductive activity for osteoprogenitor cell migration towards the implant surface (Davies *et al.* 1998).

3- Rough surface enhances mechanical interlocking between implant macromolecules and the bone at the bone-implant interface (Wennerberg *et al.* 1996, 1997).

Suggested clinical advantages of rough surfaced implants include:

- a- Shorter healing period.
- b- The use of shorter implants where vertical bone dimensions are limited (e. g. posterior mandible & maxilla).
- c- By using shorter implants invasive surgical procedures can be avoided (e. g. sinus floor lift and nerve transposition),
- d- The reduction of dental implants number.
- e- The lack of necessity of biocortical implants anchorage.

2.3 The Criteria for Implant Success

Studies have shown that the placement of endosseous implants is a predictable procedure. Several criteria have been proposed for determining the long-term success of functioning dental implants (Schnitman & Shulman 1979, Cranin *et al.* 1982 McKinney *et al.* 1984, Albrektsson *et al.* 1986, Smith *et al.* 1989).

Schnitman & Shulman (1976) described the following criteria for dental implant success:

1. Mobility less than 1 mm in any direction.
2. Bone loss no greater than one third the vertical height of the bone.
3. Gingival inflammation amenable to treatment; absence of symptoms and infection, absence of damage to adjacent teeth, absence of paraesthesia and

anaesthesia or violation of the mandibular canal, maxillary sinus, or floor of the nasal passage.

4. Functional service for five years in 75% of patients.
5. Radiologically observed radiolucency graded but no success criterion defined.

Cranian *et al.* (1982) proposed the following criteria:

1. In place 60 months or more.
2. Freedom from haemorrhage according to Muhleman's Index.
3. Lack of mobility.
4. Absence of pain or percussive tenderness.
5. No peri-cervical granulomatosis or gingival hyperplasia.
6. No evidence of a widening peri-implant space on radiograph.

McKinney *et al.* (1984) described the following criteria:

(a) Subjective criteria

1. Adequate function.
2. Absence of discomfort.
3. Patient belief that aesthetics and emotional and psychological attitude are improved.

(b) Objective criteria

1. Good occlusal balance and vertical dimension.
2. Bone loss no greater than one third of the vertical height of the implant, absence of symptoms and functionally stable for five years.
3. Gingival inflammation amenable to treatment.
4. Mobility less than 1 mm buccolingually, mesiodistally, and vertically.

5. Absence of damage to adjacent tooth or teeth and their supporting structures.
6. Absence of paraesthesia or violation of mandibular canal, maxillary sinus, or floor of nasal passage.
7. Healthy collagenous tissue without polymorphonuclear infiltration.

(c) Success criteria

1. Provide functional service for 5 years in 75% of patients.

Albrektsson *et al.* (1986) described the following criteria for dental implant success:

1. Individual unattached implant that is immobile when tested clinically.
2. Radiograph that does not demonstrate evidence of peri-implant radiolucency.
3. Bone loss that is less than 0.2 mm annually after the implant's first year service.
4. Individual implant performance that is characterized by an absence of persistent and /or irreversible signs and symptoms of pain, neuropathies, paraesthesia, anaesthesia, or violation of the mandibular canal.

In the context of the criteria mentioned a success rate of 85% at the end of a 5-year observation period and 80% at the end of 10-year observation period are minimum criteria for success.

Smith *et al.* (1989) suggested the following criteria for success:

1. The individual unattached implant is immobile when tested clinically.
2. No evidence of peri-implant radiolucency is present as assessed on an undistorted radiograph.

3. The mean vertical bone loss is less than 0.2 mm annually after the first year of service.
4. No persistent pain, discomfort, or infection attributed to the implant.
5. The implant design does not preclude placement of a crown or prosthesis with an appearance that satisfactory to the patient and dentist.

Under these criteria, a success rate of 85% at the end of a 5-year observation period and 80% at the end of a 10-year period are minimum level for success.

However, according to the recent abundance of data on marginal bone loss and a better understanding of bone and soft tissue behaviour around the implant neck and body, these criteria are inaccurate for the wide variety of implant systems. Schwartz *et al.* (2005) proposed guidelines for a novel approach to evaluate the long-term success of implants regarding marginal bone loss. Four hypothetical marginal bone loss after the first year are suggested: Low-rate marginal bone loss over the years (Albrektsson's pattern), low-rate marginal bone loss in the first few years followed by a rapid loss of bone support, high-rate marginal bone loss in the first few years followed by almost no bone loss, and continuous high-rate marginal bone loss leading to a complete loss of bone support. Correspondingly, universal success criteria should be revised.

2.4 Peri-implantitis and treatment methods

2.4.1 Clinical, Microbiological and Histopathological features

According to the first European Workshop on Periodontology (Albrektsson & Isidor 1994), peri-implantitis is defined as “inflammatory process that affects the tissues around an osseointegrated implant in function and result in loss of supporting bone”.

Results from recent studies indicate that peri-implantitis is a common disorder and the prevalence of subjects with peri-implantitis varied between 25-45% (Fransson *et al.* 2005, Ross-Jansåker *et al.* 2006b).

Clinical studies have documented that peri-implantitis may lead to implant failure and loss (Van Steenberghe *et al.* 1993, Brägger *et al.* 2001). Clinical features of peri-implantitis as described by Mombelli 1999 include the following:

- 1- Radiographic evidence of vertical destruction of the crestal bone.
- 2- Formation of peri-implant pocketing in association with radiographic bone loss.
- 3- Bleeding after gentle probing and possibly suppuration.
- 4- Mucosal swelling and redness.
- 5- Typically no pain.

Bacterial dental plaque is the primary causative factor of periodontal diseases, and this has stimulated much research to investigate the role of microorganisms in the development and progression of peri-implantitis. Lindhe *et al.* (1992) evaluated the pattern of ligature-induced periodontitis and peri-implantitis in Beagle dogs. Radiographs obtained 6 weeks after the experiment revealed substantial amount of bone loss at the tooth and implant sites. Bacterial samples demonstrated that the plaque that formed in pockets was similar at tooth and implant sites and were dominated by gram negative anaerobic bacteria. Comparable results were reported by Marinello *et al.* 1995, Ericson *et al.* 1996, Persson *et al.* 1996 and Gotfredson *et al.* 2002 who used similar models but allowing different periods of tissue breakdown.

Mombelli & Lang (1994) investigated the microbiota associated with unsuccessful implants. Samples were collected from 17 implants of various designs and analysed by phase-microscopy and in part by transmission electron microscopy. Thirteen of these implants, with stabilised pockets not exceeding 5 mm, were considered successful; 4 showed advanced pocket formation and were thus considered failures. Although the samples from the successful implants yielded a predominantly coccoid microbiota, the failed implants showed significantly elevated levels of spirochetes.

Mombelli *et al.* (1995), in a study of 10 patients with Brånemark implants and 10 patients with ITI implants, sampled the deepest residual pockets and found *P. gingivalis*, *P. intermedia*, *Fusobacterium*, and Spirochetes in many of the implant-associated deep pockets after three and six months. None of the implants was colonized by *A. actinomycetemcomitans*, but there was a similar bacterial distribution pattern for the ITI and Brånemark fixtures. However, a three-year study by Leonhardt *et al.* (1993) of 19 patients with osseointegrated implants showed a subgingival flora of predominantly *P. gingivalis*, and *A. actinomycetemcomitans*.

Studies have shown that the implants in partially edentulous patients are more at risk for peri-implantitis than those in fully edentulous patients, perhaps due to the pathogenic bacteria being transferring from the tooth pocket to the implant crevice (Meffert *et al.* 1992, Aspe *et al.* 1989, Quirynen & Listgarten 1990).

Histopathologically, peri-implantitis lesions have features that are different from those of periodontitis. The histopathologic examination of the biopsy samples from the dog study (Lindhe *et al.* 1992) revealed that there were marked differences in the size and

location of the inflammatory lesions of the two sites. Thus, while the lesion in the periodontal sites was consistently separated from the alveolar bone by a zone, about 1mm high, of non-inflamed connective tissue, the lesion in the peri-implant tissue in most situations extended into and involved the marrow spaces of the alveolar bone. This indicated that the pattern of spread of inflammation was different in periodontal and peri-implant tissues. The lesions in plaque associated periodontitis were limited to the connective tissue, while in the peri-implant tissues the lesions involved, in addition, the alveolar bone.

Histopathologic analyses of tissues sampled from peri-implantitis sites in humans revealed the presence of large inflammatory cell infiltrate in the mucosa. Sanz *et al.* (1991) analyzed soft tissue biopsies from six patients with peri-implantitis and reported that 65% of the connective tissue portion was occupied by an inflammatory cell infiltrate. Berglundh *et al.* (2003) also observed that numerous PMN cells were present in the human peri-implantitis lesions. Such cells occurred not only in the pocket epithelium and associated areas of the lesions, but also in the peri-vascular compartments distant from the implant surface.

2.4.2 Treatment of peri-implantitis in humans

Different methods have been tried and used for treatment of peri-implantitis in humans including closed debridement, open flap debridement and regenerative procedures such as membrane and/or grafts. Antimicrobial agents have been used either systemically or locally to adjunct both non-surgical and surgical therapies.

Mombelli & Lang (1992) treated 9 lesions in 9 patients by closed debridement and systemic antibiotic (Omidazole). Results showed improvement in clinical and microbiological parameters. Radiographs obtained 12 month post-operatively revealed regrowth of bone in some patients.

Mombelli *et al.* (2001) treated 30 lesions with closed mechanical debridement and placement of tetracycline fibres. Results also showed improvement in clinical and microbiological parameters. However, microbiological parameters rebounded during the 12 month observation period. Two of treated implants were subsequently lost.

Karring *et al.* (2005) studied the efficacy of closed debridement alone for treatment of peri-implantitis utilizing an ultrasonic device (Vector[®] system, Dürr Dental, Bietigheim-Bissingen, Germany) or carbon fibre curettes. Results showed no statistically significant differences for the implants treated either by ultrasonic device or manually between base line and 3 as well as 6 months, regarding bleeding on probing, PPD and radiographic bone loss. The authors concluded that closed debridement alone may not be adequate for the removal of bacterial deposit when the peri-implant pocketing depth $\geq 5\text{mm}$.

Recently Renvert *et al.* (2006) compared the combination of closed debridement and topical application of minocycline spheres with the combination of closed debridement and 1% chlorhexidine topical application. Result obtained after follow-up period of 12 months demonstrated that minocycline group showed significantly better outcomes in term of bleeding on probing and PPD reduction. In the chlorhexidine group only a limited reduction in bleeding on probing was achieved and the mean peri-implant

PPD remained unchanged. This result suggested that the topical application of chlorhexidine provides limited or no adjunctive clinical improvement compared with mechanical debridement alone.

Data for the effect of open flap debridement alone in treatment of peri-implantitis in humans are scarce. In a case series report by Leonhardt *et al.* (2003) the long term outcome of access surgery was evaluated. Resolution of peri-implant disease was obtained in 58% of the implants. However, in spite of treatment 7 of 26 implants were lost and disease progression occurred at an additional 4 implants.

In an attempt to evaluate the adjunctive decontamination effect of laser to the surgical treatment of peri-implantitis, Deppe *et al.* (2007) compared conventional decontaminated techniques with carbon dioxide laser assisted technique. Thirty two patients with 73 ailing implants included. Patients were allocated into four treatment groups:

Group 1 conventional decontamination + soft tissue resection.

Group 2 conventional decontamination + bone augmentation with beta-tricalcium phosphate.

Group 3 conventional decontamination + laser decontamination + soft tissue resection.

Group 4 conventional decontamination + laser decontamination + bone augmentation with beta-tricalcium phosphate.

Results showed that carbon dioxide laser decontamination produced better clinical outcomes than conventional decontamination especially when combined with soft tissue resection.

Several attempts have been made to regenerate bone tissue and promote osseointegration by the use of barrier membranes and/or grafts.

Jovanovic *et al.* (1993) treated 10 peri-implantitis defects using expanded polytetrafluoroethylene membranes (ePTFE) and systemic metronidazole. Result revealed significant reduction in probing depths and mean bone fill 2.3 mm.

Behneke *et al.* (2000) evaluated the effect of autogenous bone grafts for treatment of 25 peri-implantitis lesions in 17 patients. Following flap elevation and surgical curettage of the granulation tissues, fixture surfaces were cleaned by air-powder and saline and autogenous bone in the form of bone chips or bone blocks were placed and adjusted in the defects. Flaps were sutured back and the implants left non-submerged. Patients received metronidazole for 7 days. After 3 year observation period result showed improvement in clinical parameters and a mean radiographic bone fill of 4.2 mm.

In an attempt to investigate the combined effects of membrane and graft, Khoury & Buchmann (2001) treated 41 peri-implantitis lesions using autogenous bone graft alone or autogenous bone graft combined with membrane (e PTFE or collagen). Results showed no difference between the bone graft alone and the bone graft combined with either e PTFE or collagen membrane. However, in 60% of sites membrane exposure was reported.

A study by Schwarz *et al.* (2006b) evaluated the healing of intra-bony peri-implant defects following application of a nanocrystalline hydroxyapatite (NHA) paste or Bio-Oss[®] in combination with Bio-Guide[®]. Clinical parameters were reported at the base line and after 6 months. Results showed that both treatments resulted in improved clinical conditions.

Recently, a study by Ross-Jansåker *et al.* (2007a) compared two surgical techniques using a bone substitutes with or without the use of resorbable membrane. The non-submerged approach was used. As with Khoury & Buchman (2001) study, no significant differences were observed between the groups. It can be concluded that placement of membranes in addition to bone grafting does not provide any adjunctive effect.

Ross-Jansåker *et al.* (2007b) evaluated the effect of submerged approach for regenerative treatment of peri-implantitis. Sixteen implants were treated in 12 patients. Following flap elevation, granulation tissue was removed and implants surfaces were detoxified by hydrogen peroxide and irrigation with saline. Bone substitute (Algipore[®]) was placed into each defect and covered by resorbable membrane (Osseoquest[®]). Systemic antimicrobials (Amoxicillin 375 mg x 3 + metronidazole 400 mg x 2) were given for 10 days starting 1 day before surgery. Following 12 months period of observation significant clinical and radiographical improvements were observed. Mean probing depth was reduced by 4.2 mm and mean bone defect fill of 2.3 mm was obtained. The authors concluded that regenerative technique using submerged approach seemed to produce more bone defect fill than non-submerged approach by Ross-Jansåker *et al.* (2007a).

From the available literature the following conclusions may be deduced regarding treatment of peri-implantitis in humans:

- 1- Most of the available studies are case reports and case series.
- 2- Closed debridement alone may not be adequate for the removal of bacterial load from the surfaces of implants with peri-implant pockets ≥ 5 mm.
- 3- Closed debridement combined with systemic or topical antimicrobial agents can improve the condition of peri-implantitis lesions at least on a short-term basis. However, failures have been reported.
- 4- No single method of surface decontamination (chemical agents, air abrasive and laser) was found to be superior.
- 5- So far it is not known if the adjunctive use of systemic antimicrobial agents is required for surgical treatment of peri-implantitis.
- 6- Regenerative procedures such as bone graft techniques with or without the use of barrier membranes resulted in various degree of success.

2.4.3 Treatment of peri-implantitis in animal models

Various methods have been tried and used for treatment of peri-implantitis in animals including closed debridement, open flap debridement and regenerative procedures such as membranes and/or grafts. The majority of the available studies used antibiotics as an adjunct to the surgical procedures.

In most of the studies peri-implantitis was induced by sub-mucosal placement of cotton ligature. The effectiveness of ligature induction of peri-implantitis and the relative similarity of this induced lesion to naturally occurring peri-implantitis in human has been questioned. Schwarz *et al.* (2007) carried out a comparison between naturally occurring peri-implantitis defects in 24 patients with moderate to advanced peri-implantitis and ligature-induced defects around 3 implants placed bilaterally in the mandible of five beagle dogs. Following placement of the implants in the dog model, 3 months integration time was allowed during which a plaque control programme was implemented. Following ligature placement apical to the gingival margin around each implant this oral hygiene was terminated. The ligature was replaced every three weeks until 30% of the initial radiographic bone support was lost. The defect configuration and size was then evaluated following flap elevation by measuring the vertical and horizontal dehiscence components of the defect, the circumferential extent of the defect from three points (mid-buccal, mesial, distal) and the intra and supra alveolar components of the defect. The results showed a similarity between the configuration and size of ligature induced peri-implantitis and naturally occurring defects in humans. The circumferential defect without dehiscence of the adjacent alveolar crest was the most common configuration in both groups (55.3% of naturally occurring defects, 86.6% of ligature induced defects). The authors concluded that ligature induced peri-implantitis in beagle dog model is a valid representation of naturally occurring defects in humans and can be used in experimental studies investigating the treatment of peri-implantitis.

Various methods have been used for decontamination of implants surfaces e.g. air powder abrasion, saline wash, citric acid treatment, laser therapy, peroxide treatment,

ultrasonic and manual debridement and application of topical medication. The various options have associated advantages and disadvantages. Metal curettes and metallic ultrasonic tips have been shown to damage the titanium implant surface (Speelman *et al.* 1992). Superpulse laser irradiation can have adverse effects on the surface properties of lasered implants (Deppe *et al.* 2002). Collectively studies indicate all methods of surface decontamination achieve resolution of the inflammatory lesion but fail, in themselves, to achieve significant re-osseointegration along the previously contaminated implant surface (Ericsson *et al.* 1996, Persson *et al.* 1996, 1999, 2001, Shibli *et al.* 2003, Schwarz *et al.* 2006a).

Ericsson *et al.* (1996) treated 30 ligature-induced peri-implantitis lesions in 5 Labrador dogs by open flap debridement combined with systemic antibiotic. Following flap elevation and surgical curettage of the granulation tissue in one side, the fixture surfaces were decontaminated by delmopinol HCL and the flap sutured back. The implants were left non-submerged and antimicrobials (Amoxicillin and metronidazole) administered for 3 weeks starting one week before surgery. The results showed resolution of inflammation around the implants treated by local debridement, no resolution of inflammation around untreated implants, and no bone fill of the defects and no re-osseointegration. These observations demonstrated that a treatment regimen restricted to systemic antimicrobials alone is not effective in the management of peri-implantitis and that their use should be combined with meticulous removal of the biofilm from the contaminated implant surface.

Persson *et al.* (2001b) treated 8 ligature-induced peri-implantitis lesions in 4 Beagle dogs. Two types of implant were used, smooth (turned) and rough (SLA) surface

implants. Result showed resolution of peri-implantitis and bone fill in adjacent bone defects. Further, while substantial re-osseointegration occurred to an implant with rough surface (SLA), bone growth on the previously exposed machined surface was minimal.

Regenerative procedures such as bone grafting with or without membranes have been investigated by different authors.

Jovanovic *et al.* (1993) studied the regenerative potential of plaque-induced peri-implant bone defects treated by submerged membrane technique in beagle dogs. Plaque accumulation was achieved by tying silk ligature around the coronal implant portion. Implant surfaces were machined, plasma-sprayed or hydroxyapatite-coated. After 3 months of plaque accumulation and bone defect formation, a total of 21 implants (7 of each type of implant surface) formed the experimental group and 9 implants served as controls. Test sites were treated with a PTFE membrane over the defect and submerged after the implant surface was decontaminated using an air powder abrasive unit and supersaturated citric acid. The result at the end of the evaluation period (2-4.5 months) showed minimal bone gain or rather fibrous soft tissue formation in those implants with membrane exposure whereas test sites in which a submerged membrane position was maintained, demonstrated complete regeneration of the peri-implant bone defect.. Some re-osseointegration was occurred in test sites but there was no re-osseointegration in the control sites.

Grunder *et al.* (1993) studied the effect of guided bone regeneration in treatment of experimentally induced peri-implantitis in dogs. After flap elevation and surgical

curettage of the granulation tissue, the implant surfaces were cleaned by an air-powder abrasive unit. Four groups of treatment were randomly assigned:

- Submerged implants treated with open flap debridement alone.
- Submerged implants treated with membranes.
- Non-submerged implants treated with open flap debridement alone.
- Non-submerged implants treated with membranes.

The result showed minimal bone fill or continued bone loss and there were no differences in outcomes between the four groups.

Persson *et al.* (1996) evaluated the effect of guided bone regeneration in treatment of experimentally induced peri-implantitis in dogs. Following flap elevation and surgical removal of the granulation tissue in the left side of the mandible, the exposed outer surface and the internal part of the fixtures were carefully cleaned with delmopinol HCL. An e PTFE membrane was placed over each fixture and adjusted to cover the bone crater. New screws were fitted through the membrane to the cleaned fixture. The implants were submerged and the flaps sutured. In the right side of the mandible no local treatment was performed. The dogs were sacrificed after 4 months and biopsies prepared for histological examination. The results showed elimination of the inflammatory process in the peri-implant tissue and no re-osseointegration.

Hurzeler *et al.* (1997) studied the effects of membranes alone, bone grafts alone and their combination in treatment of ligature-induced peri-implantitis lesions in beagle dogs. Following flap elevation and surgical curettage of the granulation tissue, the implant surfaces were cleaned with an air powder abrasive unit. The saucer- shaped bony defects were then measured and 6 different defect treatments randomly assigned:

- Debridement alone.
- Debridement plus hydroxyapatite (HA)
- Debridement plus canine demineralised freeze-dried bone (DFDB).
- Debridement plus guided bone regeneration (GBR) using e PTFE membrane.
- Debridement plus GBR and HA..
- Debridement plus GBR and DFDB.

The implants were submerged and metronidazole was administered for 3 weeks. After a healing period of 4 months all sites were reopened for clinical measurement and assessment of bone regeneration. Result showed better bone fill of the defects with membranes followed by grafts. Open flap debridement alone showed the least bone fill. However, there was no difference between membrane use and membrane plus bone grafts. Histomorphometric analysis showed better re-osseointegration with membranes followed by bone grafts followed debridement alone. Membranes combined with grafts showed significantly more re-osseointegration compared to membranes alone.

Schou *et al.* (2003a) studied the effects of membranes combined with grafts for treatment of experimentally induced peri-implantitis in monkeys. Following flap elevation and surgical curettage of the granulation tissue, the implant surfaces were cleaned with cotton soaked alternately in chlorhexidine and saline. Four treatment groups were assigned as follow:

- Debridement alone.
- Debridement plus e PTFE membrane.
- Debridement plus autogenous bone graft.
- Debridement plus autogenous bone graft plus e PTFE membrane.

The implants were non-submerged and metronidazole was administered for 12 days. Sites treated with autogenous bone graft plus membrane resulted in significantly better bone regeneration and re-osseointegration than the other techniques. Bone graft alone and e PTFE alone were better than open flap debridement alone in respect of bone fill and re-osseointegration.

The effect of implant surfaces on bone regeneration and re-osseointegration had been investigated by many authors.

Shibli *et al.* (2003) highlighted the influence of different implant surfaces on bone regeneration and re-osseointegration when using expanded polytetrafluoroethylene (e PTFE) membranes and found the greatest histological evidence of regeneration associated with Hydroxyapatite (HA) coated implants and the least for commercial pure titanium implants (cpTi). Regarding re-osseointegration, the best result was seen at titanium plasma sprayed (TSP) surfaces. Nevertheless, small sample size should be considered.

Wetzel *et al.* (1999) evaluated the effects of different implant surfaces on bone defect fill and re-osseointegration. Four types of implant surfaces were investigated; titanium plasma sprayed implants (TPS), TPS-furcation implants, sand-blasted acid etched implants (SLA) and machined surface implants (M). Result showed a significant differences in terms of bone defect fill and re-osseointegration between different implant surfaces with sand-blasted acid etched (SAL) being superior followed by TPS and then machined surface implants.

Recently Persson *et al.* (2004) and Sennerby *et al.* (2005) both demonstrated that sand-blasted and acid etched implants were superior to machined surface implants in term of regeneration.

In an attempt to compare the surgical with non-surgical treatment Schwarz *et al.* (2006a) evaluated non-submerged and submerged healing of ligature induced peri-implantitis in dogs. Peri-implantitis was induced by ligature placement around 30 implants in five beagle dogs. The defects were randomly and equally allocated in a split-mouth design to either closed treatment + non-submerged healing (CNS) or open treatment + submerged healing (OS) using an ER:YAG laser (ERL), an ultrasonic device (VUS), or plastic curettes + local application of metronidazole gel (PCM). The dogs were sacrificed after 3 months. All treatment procedures resulted in statistically significant improvement of all clinical parameters. Radiological improvements were merely observed at OS implants. Histomorphometric analysis revealed that all CNS implants exhibited comparable low amount of new bone to implant contact (1.0-1.2%). While mean bone to implant contact was statistically significant higher in the respective OS groups (ERL 44%, PCN 14.8% and VUS 8.7%). The authors concluded that open treatment with submerged healing has better outcome compared to closed treatment with non-submerged healing and ERL seemed to be more suitable to promote re-osseointegration than PCN and VUS.

Surgical implantation of growth factors such as bone morphogenetic protein-2 (BMP-2) and platelets growth factors had been emerged as a possible treatment of peri-implantitis as well. Hanisch *et al.* (1997) evaluated bone formation and re-osseointegration following surgical implantation of recombinant human bone

morphogenetic protein- 2 (rhBMP-2) in peri-implantitis defects. Hydroxyapatite coated dental implants were placed bilaterally in mandibular and maxillary premolar area of 4 rhesus monkeys and were allowed to osseointegrate for 1 year. Peri-implantitis was induced by cotton ligature placement apical to the gingival margin. The peri-implantitis defects were randomly assigned to receive rhBMP-2 in absorbable collagen carrier or a carrier control. Results showed that vertical bone gains as well as re-osseointegration were greater in rhBMP-2 defects than in control defects. The authors concluded that rhBMP-2 has potential to promote bone formation and re-osseointegration in advanced peri-implantitis defects in nonhuman primate model.

Recently, You *et al.* (2007) studied the treatment of experimental peri-implantitis using autogenous bone grafts and platelet-enriched fibrin glue in dogs. Thirty-six screw type commercially pure titanium implants with rough acid-etched surfaces were inserted in six mongrel dogs 3 months after extraction of mandibular premolars. After 3 months of healing, peri-implantitis was induced by placing gauze and wire around the implants. Two-four months following ligature placement the resulted bone defects were assigned to three treatment groups as follow:

- Open flap surgery + combination of autogenous bone grafts and platelet-enriched fibrin glue.
- Open flap surgery + autogenous bone grafts alone.
- Open flap surgery alone (control).

Six months after healing, biopsies of the implant sites were taken and prepared for analysis. Results revealed that peri-implantitis defects treated with combined autogenous bone grafts and platelet-enriched fibrin glue showed significantly higher

re-osseointegration compared to the other 2 treatment procedures. A mean bone-to-implant contact was 50.1%, 19.3% and 6.5% for defects treated with the combined autogenous bone grafts and platelet-enriched fibrin glue, autogenous bone grafts alone and surgery alone respectively. The authors concluded that surgical treatment involving the combined use of autogenous bone grafts and platelet-enriched fibrin glue might effectively promote re-osseointegration in lesions resulting from peri-implantitis.

From the available literature the following conclusions may be deduced regarding treatment of peri-implantitis in animal models:

- 1- Sub-mucosal placement of cotton ligature is the most common technique that used to induce peri-implantitis in animals.
- 2- Majority of studies utilized primary flap closure and post-operative submerging of the treated defects/implants.
- 3- Open debridement including surface decontamination was more effective than closed debridement in treatment of peri-implantitis.
- 4- Open flap debridement including surface decontamination resolved peri-implantitis, enhanced bone defect fill and may result in re-osseointegration. However, re-osseointegration was more pronounced on rough surfaces than on smooth surfaces implants.
- 5- No single method of surface decontamination was found to be superior.
- 6- Regenerative technique using bone grafting with or without barrier membranes resulted in varying amount of bone defect fill and re-osseointegration.

2.5 Osseointegration of implants surrounded by bony defects

Bone loss associated with peri-implantitis results in a gap between the implant surface and the surrounded bone. Such gap may influence re-osseointegration following treatment of peri-implantitis. The influence of dimension and configuration of bone defect at dental implants regarding bone fill and osseointegration had been investigated in different studies.

Carlsson *et al.* (1988) placed titanium rods of varying widths into tibias of rabbits. After 6 and 12 weeks of healing, histologic evaluation revealed no direct bone-to-implant contact in 10 out of 13 implants when the initial gap between the bone and implant had been larger than 0.35 mm.

Caudil & Meffert (1991) studied the effect of bony defects around dental implants in mongrel dogs. Simulated extraction sockets were prepared on each side of the mandible. Hydroxyapatite-coated implants were placed into the prepared sockets in such a way that a 1 mm bony defect surrounded the coronal part of each implant. Half of the implants received overlying barriers membranes. After the 9 weeks healing period, the results demonstrated that the apical “intimate fit” portion of each implant had osseointegrated at the light microscope level, while the coronal portions did not have bone contacting the implant. The authors suggested that a possible cause of osseointegration failure at the coronal portion due to insufficient healing time and the available vertical bone height allowed only half of the length of the implant in bone.

Knox *et al.* (1991) studied the effect of the gap around dental implants in six mongrel dogs. Four standard implant sites, 3.25 X 10 mm, were prepared bilaterally in the

mandible of each dog. One site on each side remained as control. The remaining three sites on each side were enlarged at their coronal 4 mm to create circumferential defects of 0.5 mm, 1 mm and 2 mm. After 8 weeks of healing, the result showed that implants surrounded by a gap of 0.5 mm had values similar to those of the controls for the level of osseointegration and residual defect area. The implants surrounded by defects of 1 mm or 2 mm demonstrated more apical level of osseointegration and larger residual defect areas compared to the controls. The authors concluded that implants surrounded by bony defects on their coronal portions can osseointegrate; however the level of osseointegration is influenced by the width of the bony defect.

Akimoto *et al.* (1999) evaluated the effect of gap width on bone healing around implants placed into simulated extraction socket defects of various widths in dogs. Four osteotomy sites, 2.7 mm wide and 10 mm long, were prepared in right and left sides of the mandible of each dog. One site in each side remained as a control. Control sites received 3.3 mm implants. In the test sites, the coronal 6 mm portion was enlarged to 4.3 mm, 5.25 mm and 6 mm. Test sites received 3.3 mm implants. The modifications in the test sites created 0.5 mm, 1 mm and 1.4 mm gap around the implants in the test sites. Histomorphometric analysis revealed that for the coronal 6 mm of the implants the control sites demonstrated the highest mean percentage of bone-to-implant contact (38.8%), followed by the sites with 0.5 mm gaps (22.9%), by the sites with 1 mm gaps (11.1%) and by the sites with 1.4 mm gaps (2.7%). In the apical 4 mm of the implants, no statistical differences in percentage of bone-to-implant contact were found. The authors concluded that osseointegration at the implant surface surrounded by bone defect is influenced by the width of the bone

defect, with small diameter defects showing better osseointegration than larger defects.

Botticelli *et al.* (2004) investigated bone healing at implant sites with hard tissue defects of varying dimensions and configurations. Four Labrador dogs were used. Three months after extraction of all mandibular premolars and first molars five implants with sand blasted, large grit, and acid etched (SLA) surfaces were placed as follow:

In the first premolar sites the implants were placed in intact bone, in the second premolar sites circumferential gap 1 -1.25 mm wide was created at the coronal 5 mm part of each implant, in the third premolar sites circumferential gap 1 -1.25 mm wide was created at the coronal 5 mm part of each implant and the buccal wall removed, in the fourth premolar sites circumferential gap 2 -2.25 mm wide was created at the coronal 5 mm part of each implant and in the first molar sites Circumferential gap 2 - 2.25 mm wide was created at the coronal 5 mm part of each implant and the buccal wall removed. Results showed that four wall defects of different dimensions (1-2.25 mm wide) were resolved during healing. Further, at sites where the buccal wall was removed intentionally, healing resulted in defect resolution at the mesial, distal, and lingual aspects. At the buccal aspect healing was incomplete but the dimension of the defect was reduced by limited amount of new bone formation extending from the lateral and apical borders of the defect.

In an attempt to evaluate the influence of the surface texture on osseointegration at implant surface surrounded by bone defect Botticelli *et al.* (2005) compared bone healing at implants with turned or rough surface topographies placed in self-contained

defects either submerged or non-submerged. Six dogs were used. Three months after tooth extraction four implants were placed in each side of the mandible (Two rough surfaces and two turned surfaces). The implant in the right side was fully submerged and those in the left side were non-submerged. A circumferential gap between 1 and 1.25 mm was created around the marginal 5 mm part of each implant. The animals were sacrificed 4 months later. Results demonstrated that bone defects around rough surface implants either submerged or non-submerged exhibited substantial bone fill and high degree of osseointegration. Healing at turned implants was characterized by incomplete bone fill and the presence of connective tissue zone between the implant the newly formed bone. The authors concluded that osseointegration at implants placed in sites with marginal defects is influenced by the surface characteristic of the implant.

The influence of surface characteristic on osseointegration of implants placed in sites with bone defects had been further documented by Salata *et al.* (2007). The authors compared the integration and implant stability of turned and oxidized titanium implants when placed in experimental bone defects with autogenous bone graft, BMP-2 or without adjunctive therapy. Results showed that oxidized implants showed a significantly higher stability and more bone contact to implant than turned surface implants. The authors concluded that oxidized implants gain stability more rapidly and integrate with more bone contacts than implants with turned surface when placed in bone defects.

Jung *et al.* (2007) evaluated the healing of the surgically created circumferential bone gaps around submerged-type implants in mongrel dogs. According to the width of the

gap implants were allocated into three groups, 1.0 mm group, 1.5 mm group and 2.0 mm group. Results showed that as the size of the coronal gap increased, the unfilled area tended to be greater. Both 1.0 mm and 1.5 mm gaps showed large percentage of osseointegration of the coronal defect than the apical side, while the 2.0 mm gap showed large percentage of osseointegration at the apical side than the coronal defect. The authors concluded that the remaining defect, small enough to be clinically neglected (within 2.0 mm) does not need any kind of regenerative procedure. Adjunctive effects of different bone grafting materials have been evaluated regarding osseointegration of implants placed in sites with bone defects.

Polyzois *et al.* (2007) studied the effect of grafting materials on osseointegration of dental implants surrounded by circumferential bony defects of different dimensions in dogs. Results showed that bone grafting with autogenous or bovine anorganic cancellous bone xenograft lead to a more favourable histological outcome for wider (2.37mm) circumferential defects than for narrower defects (1.0mm). However, there was no significant difference of osseointegration between non-grafted 1.0mm circumferential defects sites and the control sites. This result indicated that osseointegration can occur around implants surrounded by non-grafted 1 mm circumferential bony defects.

Abushahba *et al.* (2008) studied the effects of different grafting materials on osseointegration of dental implants surrounded by 1.35 mm circumferential bone defects in beagle dogs. Results revealed that autogenous bone graft or Bio-Oss® seems to lead to more favourable histological outcomes. Osseointegration and bone regeneration were significantly better in augmented sites as compared to defects

without augmentation. There was no significant difference of osseointegration between non-grafted sites and the control (no bone defects).

So far in animal models studies have shown that contamination of the implant surface will not prevent osseointegration (Kolonidis *et al.* 2003, Alhag *et al.* 2008). Furthermore, studies have shown that the amount of osseointegration around dental implants placed in sites with bone defects is influenced by the width of the defect. (Knox *et al.*1991, Akimoto *et al.*1999, Polyzois *et al.* 2007). The aim of this study was to investigate the effect of surface contamination on re-osseointegration of dental implants surrounded by circumferential bone defect of 1.0 mm and to compare the amount of osseointegration around Osseotite[®] implants (Biomet 3I, Palm Beach Gardens, USA) with that around Nanotite[™] implants (Biomet 3I, Palm Beach Gardens, USA) in beagle dogs.

Authors (year)	Number of implants/ patients	Type of implant	Treatment	Observation period	Treatment outcome
Mombelli & Lang (1992)	9 implants/ 9 patients	Titanium hollow cylinder impl. (ITI type F or Bonefit)	Closed debridement+ systemic ornidazole 1000 mg/ day for 10 days	12 months	Clinical: BI: 1.6-0.7 (P< 0.01) PD: 5.9-3.4 mm (p< 0.001) Microbiological: G - anaerobic rods: 40%-16% Radiographic: Regrowth of bone in some patients
Jovanovic et al. (1992)	10 implants/7 patients	6 IMZ implants. 3 Brånemark implants. 1 TPS implant	Open flap debridement+ e-PTFE + systemic tetracycline 250 mg four times/day for 7 days	6 months	Clinical: PI: 1.7-0.6 GI: 2.1-0.3 PD: 6.8-4.1 mm Radiographic: 7 defects: Evidence of excellent repair with bone 3 defects: Did not demonstrate any defect fill
Behneke et al. (2000)	25 implants/17 patients	ITI implants	Open flap debridement + autogenous bone graft + systemic metronidazole 400 mg twice/day for 7 days	6 months-3 years	Clinical: (1 year/18 implants) PD: 5.3-2.2 mm (3 years/10 implants) PD: 5.3-1.6 mm Radiographic: (1 year/18 implants) 3.9 mm (3 years/ 10 implants) 4.2 mm
Mombelli et al. (2001)	30 implants/ 25 patients	ITI (Bonefit)	Closed debridement + tetracycline fibres for 10 days	12 months	Clinical: PI: 0.22-0.15 BI: 0.95-0.37 PD: 4.7-3.5 mm Microbiological Total counts: 3.4-3.1 x 10 ⁶ G- anaerobic rods: 1.6-1.5 x 10 ⁶ (rebound between 6-12 months) Radiographic: 0.3 mm bone fill

Cont. Table 2.1. Human studies for treatment of peri-implantitis

Khoury & Buchmann (2001)	Group 1: 12 implants/7 patients Group 2: 20 implants/11 patients Group 3: 9 implants/7 patients	IMX and Friadent (F2) implants	G1: Open flap debridement + autogenous bone graft G2: Open flap debridement + autogenous bone graft + e- PTFE G3: Open flap debridement + autogenous bone graft + collagen membrane	3 years	Clinical: G1: PD: 6.5-2.9 mm G2: PD: 6.7-2.8 mm G3: PD: 6.4-5.1 mm Radiographic: G 1: 2.2 mm bone fill G 2: 2.5 mm bone fill G 3: 1.7 mm bone fill
Leonhardt et al. (2003)	26 implants/ 9 patients	Brånemark implants	Open flap debridement + systemic antibiotic	60 months	Healing was obtained in 58% of the implants. 7 implants were lost.
Karring et al. (2005)	22 implants/11 patients	Brånemark [®] , Straumann [®] , Astra [®]	G1: closed debridement with Vector [®] ultrasonic device G2: Closed debridement with carbon fibre curettes	6 months	No statistically significant differences for either groups between the baseline and 3 as well as 6 months, regarding bleeding on probing, PPD and radiographic bone loss
Renvert et al. (2006)	87 implants/ 30 patients	Brånemark implants	G1: closed debridement + minocycline microsphere G2: closed debridement + chlorhexidine 1%	12 months	G1: PD: 3.9-3.6 mm G2: PD no change
Schwarz et al. (2006b)	22 implants/ 22 patients	Brånemark, Straumann, KSI Bauer, MTX, TSV and ZL-Duraplast	G1: Access flap surgery + nanocrystalline hydroxyapatite G2: Access flap surgery + bovine derived xenograft and collagen membrane (Bio-Gide [®])	6 months	G1: PD: 7-4.9 mm CAL: 7.5- 5.7 mm G2: PD: 7.1-4.5 mm CAL: 7.5- 5.2 mm

Ross-Jansåker et al. (2007a)	65 implants/36 patients	Brånemark(36) machined surface, Astra (2) rough surface	Group 1: Open flap debridement + bone substitute (Algipore®) +resorbable membrane (Osseoquest®) Group 2: Open flap debridement + bone substitute (Algipore®) Systemic antibiotics (amoxicillin 375 mg x 3 + metronidazole 400 mg x 2) given to the two groups for 10 days	12 months	G1: PD was reduced by 2.9 mm and a mean defect fill of 1.5 mm was obtained G2: PD was reduced by 3.4 mm and a mean defect fill of 1.4 mm was obtained
Ross-Jansåker et al. (2007b)	16 implants/ 12 patients	Brånemark	Open flap debridement + bone substitute (Algipore®) + Resorbable membrane (Osseoquest®) (Submerged) Systemic antibiotics (amoxicillin 375 mg x 3 + metronidazole 400 mg x 2) for 10 days	12 months	Clinical and radiographic improvements were observed. PD was reduced by 2.4 mm and mean defect fill of 2.3 mm was obtained

PI; plaque index, BI; bleeding index, CAL; clinical attachment level, G; Group, PD; probing depth, e-PTFE; polytetrafluoroethylene membrane,

Table. 2.2 Animal studies for treatment of peri-implantitis

Authors (year) Animals	Number of implants/ animals	Type of implant	Treatment	Observation period	Treatment outcome
Grunder et al. (1993) Beagle dogs	40 implants/10 animals	Titanium implants (Screw-vent, Dentsply)	G1: access flap surgery (submerged implants) G2: access flap surgery + e-PTEF (submerged) G3: access flap surgery (non-submerged) G4: access flap surgery + e-PTEF (non- submerged)	12 months	Bone fill: G1: 0.3 mm G2: -0.1 mm G3: 0.2 mm G4:-0.1 mm Re-osseointegration: No
Jovanovic et al. (1993) Beagle dogs	30 implants/ 3 animals	Brånemark, IMZ and HA –coated impl. (Integral, calcitec)	G1: Access flap surgery (submerged) G2: Access flap surgery + e-PTEF (submerged)	2/4.5 months	Bone fill: G1: minimal bone formation G2: 15 implants showed complete bone defects closure Re-osseointegration: G1: no G2: some
Ericsson et al. (1996) Labrador dogs	30 implants/5 animals	Brånemark system, Nobel- pharma)	Test group: Access flap surgery Control group: No treatment The two groups given amoxicillin + metronidazole for 3 weeks.	4 months	Bone fill: No Re-osseointegration: No
Persson et al. (1996) Labrador dogs	30 implants/ 5 animals	Brånemark system, Nobel- pharma	Test group: Access flap surgery + e-PTEF Control group: No treatment. The two groups given amoxicillin + metronidazole for 3 weeks.	4 months	Bone fill: No Re-osseointegration: No

Cont. Table. 2.2 Animal studies for treatment of peri-implantitis

Hürzeler et al. (1997) Beagle dogs	42 implants/ 7 animals	Brånemark, Nobel Biocare	G1: Access flap surgery G2: Access flap surgery +HA G3: Access flap surgery +DFDB G4: Access flap surgery +e-PTFE G5: Access flap surgery +HA+ e-PTFE G6: Access flap surgery + DFDB+ e-PTFE	5 months	Bone fill: G1: 0.5 mm G2: 1.3 mm G3: 1.6 mm G4: 2.5 mm G5: 2.4 mm G6: 3.0 mm Re-osseointegration: G1: 0.3 mm G2: 0.9 mm G3: 0.9 mm G4: 1.0 mm G5: 2.3 mm G6: 2.2 mm
Hanisch et al. (1997) Rhesus monkey	31 implants/ 4 animals	Bio-Vent, Dentsply	G1: Access flap surgery + rh BMP-2 G2: Access flap surgery + vehicle control	4 months	Bone fill: G1: 2.6 mm G2: 0.8 mm Re-osseointegration: G1: 40% G2: 9%
Wetzel et al. (1999) Beagle dogs	39 implants/ 7 animals	ITI, Straumann	G1: Access flap surgery G2: Access flap surgery +e-PTFE	6 months	Bone fill: G1: 0.3-0.8 mm G2: 2.2-2.6 mm Re-osseointegration: G1: 0.2-0.3 mm G2: 0.1-0.6 mm

Cont. Table. 2.2 Animal studies for treatment of peri-implantitis

Persson et al. (2001b) Beagle dogs	8 implants/ 4 animals Machined and rough surfaced implants	ITI,Straumann	Access flap surgery (submerged) Amoxycillin + metronidazole for 17 days	6 months	Bone fill: Machined: 72% of the defect area filled with bone Rough: 77% of the defect area filled with bone Re-osseointegration: Machined: 63% Rough: 69%.
Schou et al. (2003a) Monkeys	64 implants/ 8 animals	Tps	G1: Access flap surgery G2: Access flap surgery +autogenous bone graft G3: Access flap surgery +e-PTFE G4: Access flap surgery +autogenous bone graft + e-PTFE	6 months	Bone fill: radiographic and histologic results revealed almost total bone fill in defect irrespective of treatment Re-osseointegration: Mean bone to implant contact of 39-46% irrespective of treatment.
Shibli et al. (2003) Mongrel dogs	36 implants/ 6 dogs	Cp Ti HA TPS Hybrid (machined and acid etched)	Surface rinsed with physiologic saline, Lethal photosensitization and e-PTFE membrane	5 months	Bone fill: HA: 48.28% TPS: 39.54% AE: 26.88%, cp Ti 26.7% Re-osseointegration: HA: 15.83% TPS: 25.25% AE:17.3% Cp Ti: 24.94%

Cont. Table. 2.2 Animal studies for treatment of peri-implantitis

Schwarz et al. (2006a) Beagle dogs	30 implants/ 5 animals	Straumann implants	G1: Er; YAG laser, closed debridement, non- submerged G2: Er; YAG laser, open flap debridement, submerged G3: Ultrasonic, closed debridement, non- submerged G4: Ultrasonic, open flap debridement, submerged G5: Plastic curette + topical metronidazole, closed debridement, non- submerged G6: Plastic curette + topical metronidazole, open flap debridement, submerged	3 months	Bone fill: Surgical groups: G2: 1.3 mm G4: 0.5 mm G6: 0.9 mm Re-osseointegration: G2: 44.8% G4: 14.8% G6: 8.7% Non-surgical groups: Bone regeneration range 15% to 23% Re-osseointegration: range 1% to 1.2%
You et al. (2007) Dogs	36 implants/ 6 animals	Cpi T Screw type implants	G1: debridement alone G2: debridement + autogenous bone graft G3: Debridement + autogenous bone graft + platelet enriched fibrin	6 months	Bone fill: Thin connective tissue capsule separating bone from implant surface in all groups Re-osseointegration: G1: 6.5% G2: 19.3% G3: 50.1%

PI; plaque index, BI; bleeding index, CAL; clinical attachment level, G; Group, PD; probing depth, e-PTFE; polytetrafluoroethylene membrane,

CHAPTER 3

MATERIALS AND METHODS

3.1 Implants

Eight commercially pure titanium 3.25 mm x 13 mm Osseotite[®] (Biomet 3I, Palm Beach Gardens, USA) and eight commercially pure titanium 3.25 mm x 13 mm Nanotite[™] (Biomet 3I, Palm Beach Gardens, USA) implants were used.

3.2 Animals and Anaesthesia

Four adult beagle dogs were used in this study. During surgical procedures the anaesthesia were induced with thiopentone; 2.5% solution, 20mg/Kg intravenously and maintained with halothane.

3.3 Extractions

A local anaesthetic (Xylocaine[®] 2% with adrenaline 1:80,000, Astra AB, Södertälje, Sweden) was administrated to the areas undergoing surgeries to control bleeding. Full-thickness mucoperiosteal flaps were reflected. High speed carbide burs were used to hemisect positions P2, P3 and P4 at furcation. The roots were separated and removed. The single rotted P1 was also extracted. The surgical sites were sutured with black silk sutures. Sutures were removed after seven days. The animals were kept for a healing period of four months.

3.4 Experimental Procedures

3.4.1 Experimental Procedure 1

Following the healing period, general anaesthesia was again administered as described earlier. An incision was made at the crest of the lower ridges from the canine to the first molar. Full thickness buccal and lingual mucoperiosteal flaps were reflected to a

level approximating the lower border of the mandible. The neurovascular bundles from both mental foraminae were identified and protected. Four osteotomies were prepared using standard drilling procedure and two 3.25 mm x 13mm narrow-platform Osseotite[®] and two 3.25 mm x 13mm Nanotite[™] were partially inserted in the left side of each mandible. The flaps were repositioned and sutured using intercepted sutures with 4/0 black silk. The sutures were removed after 7 days. Plaque accumulation was facilitated by maintaining the dogs on a soft diet for a healing period of five weeks.

3.4.2 Experimental Procedure 2

Following the five weeks healing period, general anaesthesia was again administered as described earlier. The numbers of threads exposed to the oral cavity for each implant were counted.

Full- thickness buccal and lingual mucoperiosteal flaps were raised and reflected on the left side of each mandible and the implants surgically exposed to bone level. The numbers of threads protruding from bone for each implant was counted. The implants were removed by screwing them anticlockwise. Following removal of the implants, the alveolar bone crest was re-contoured with a large round bur to minimize irregularities and so minimize postoperative discomfort to the animals. The flaps were sutured with 4/0 black silk interrupted sutures. The contaminated parts of each implant were treated using mechanical cleansing with a tooth brush and physiological saline for 1 minute (Dennison *et al.* 1994).

On the contralateral sides of the mandibles, full thickness flaps were raised on both buccal and lingual aspects to a level approximating the lower border of the mandible and the neurovascular bundles from both mental foraminae were identified and protected. The four contaminated implants were placed into freshly prepared sites to

the full implant length on the right side of each mandible. The apical 8mm of each osteotomy site was prepared with 2.75mm twist drill and the coronal 5mm with a 5.25mm twist drill. This resulted in approximately 1.0mm circumferential defects. The flaps were sutured using 4/0 black silk interrupted sutures to achieve a complete wound closure. The sutures were removed after seven days. The dogs were reinstalled in kennels and kept there for a healing period of eleven weeks. The distribution of the implants by site is shown in Table 1.

Table 3.1 Distribution of the implants by site

	LP1	LP2	LP3	LP4
DOG 1				
TYPE	1	1	2	2
DOG 2				
TYPE	1	2	2	1
DOG 3				
TYPE	2	2	1	1
DOG 4				
TYPE	2	1	1	2

- LP1 Lower first premolar
- LP2 Lower second premolar
- LP3 Lower third premolar
- LP4 Lower fourth premolar

Implant type:

1. Osseotite[®]
2. Nanotite[™].

3.5 Antibiotics and Follow-up

After the surgical procedures all the dogs were given 150 mg oral Clindamycin twice daily for 7 days and buprenorphine(0.02mg/Kg) as needed for pain as determined by their activities.

3.6 Specimens Preparation

After 12 weeks of healing the dogs were sacrificed by intravenous injection of 200 mg/kg of pentobarbitone. The mandibles on the experimental sides were harvested, cleaned of all soft tissue and fixed in 10% buffered formalin. Each implant site was separated using a diamond saw (Exact[®], Kulzer, Germany). The specimens were then rinsed in water, dehydrated in ethanol, and preinfiltrated with dilute methylmethacrylate. They were then infiltrated with pure methylmethacrylate and allowed to polymerise over 14 days.

3.7 Histological Preparation

The blocks were then sectioned mesio-distally, ground to 100µm, and stained with 1% toluidine blue. Slides were obtained from the blocks and analyzed.

3.8 Measurement Method

The histological slides were magnified under light microscopy by 12.5 times and images were captured with an Optronic CCD digital Microscope Camera and saved on computer hardware. Analysis software (Scion Image Beta 4.02 Win, Scion PCI Frame Grabber boards) was calibrated to present all the measurements from the histological slides in millimetres.

3.9 Histometric Examination

Two measurements were made:

- 1- Linear measurement of bone in direct contact with the implant surface. The bone in direct contact to the implant surface was measured in both mesial and distal aspect of each implant section. The two measurements were added together and the percentage of bone in direct contact to implant surface was calculated
- 2- Area measurement of bone formation within the threads of the implant. The bone within the threads of the implant was measured in both mesial and distal aspect of each implant section. The two measurements were added together and the percentage of bone within threads was calculated.

3.10 Data Analysis

Regression models were used to analyze the effects of implant site, part and type on the two measurements. The independent variables were; implant site - implant site 1; lower 1st premolar, implant site 2; lower 2nd premolar, implant site 3; lower 3rd premolar, implant site 4; lower 4th premolar, the Implant type – Type 1; Osseotite[®], Type 2; Nanotite[™] and the implant part- the part that previously exposed to oral environment, the part that previously covered by soft tissues and the part that surrounded by bone.

Table 3.2 Number of threads that were exposed to oral cavity.

Dog 1				
A	2	2	3	2
B	6	5	5	6
Dog 2				
A	2	2	2	3
B	6	6	5	5
Dog 3				
A	3	3	3	3
B	5	5	5	5
Dog 4				
A	3	3	4	3
B	5	5	5	5

A In the mouth before flap elevation

B After flap elevation.



Figure 3.1 Edentulous premolar areas prior to surgery.

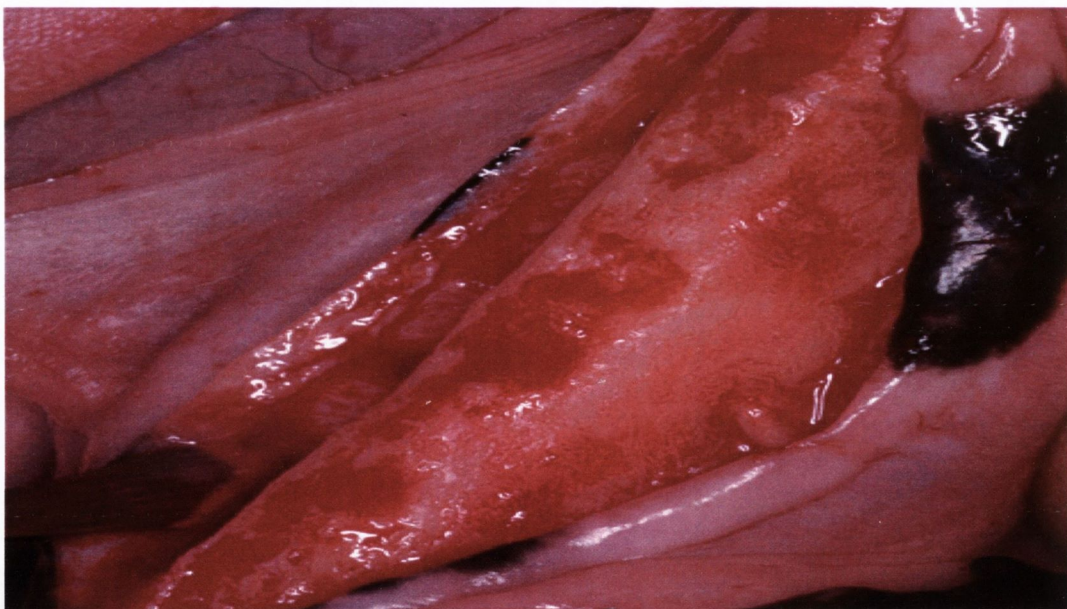


Figure 3.2 The ridge following flap elevation

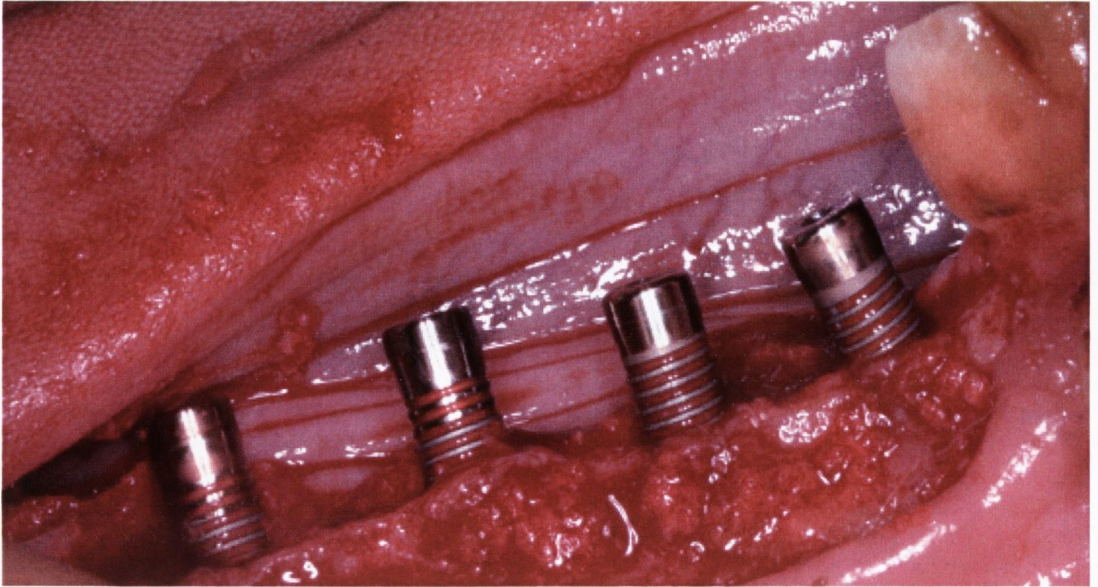


Figure 3.3 Four implants partially inserted.

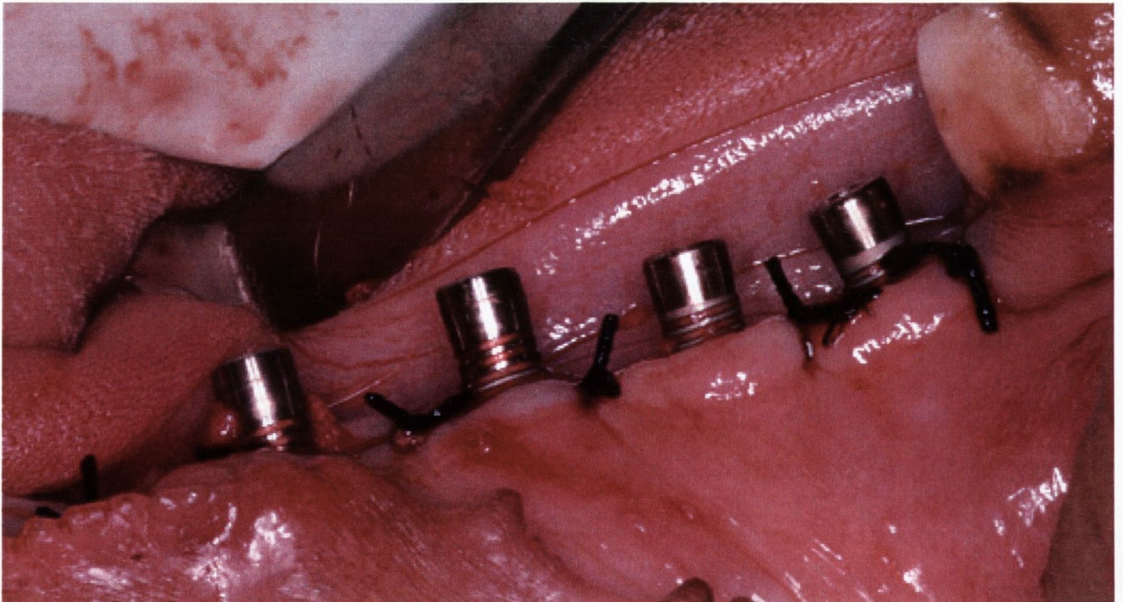


Figure 3.4 Four implants after flap sutured back.

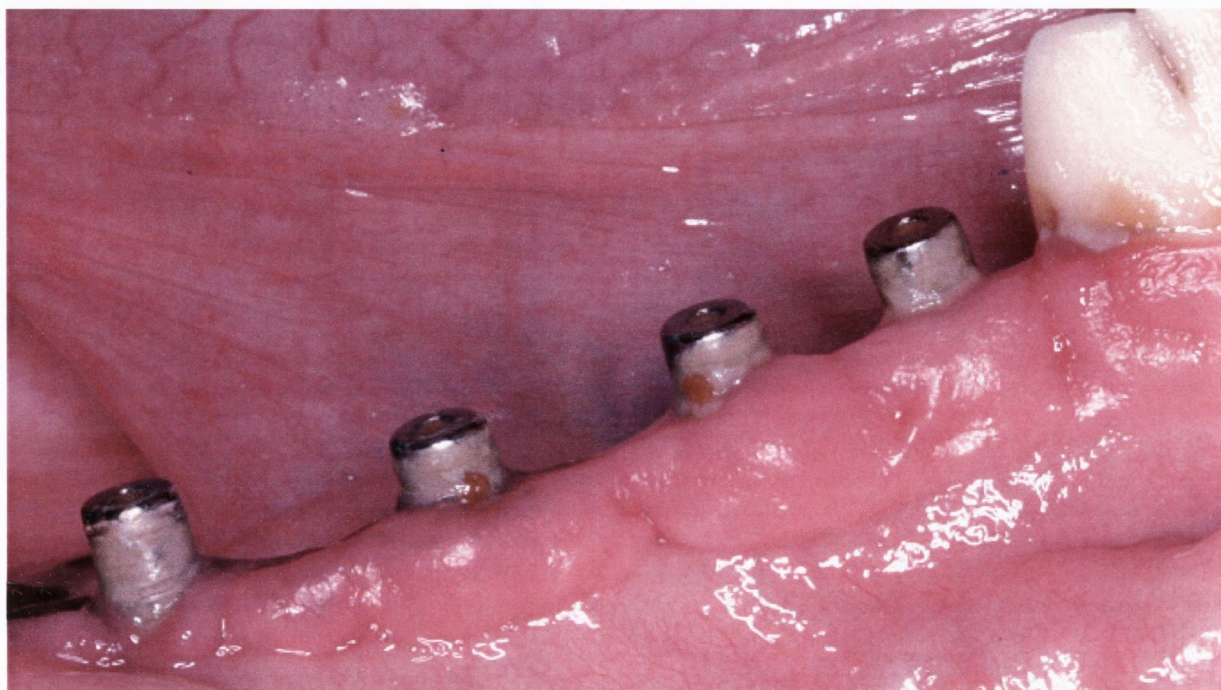


Figure 3.5 Four implants contaminated with bacterial plaque.



Figure 3.6 Implant cleaned with saline and tooth brush.

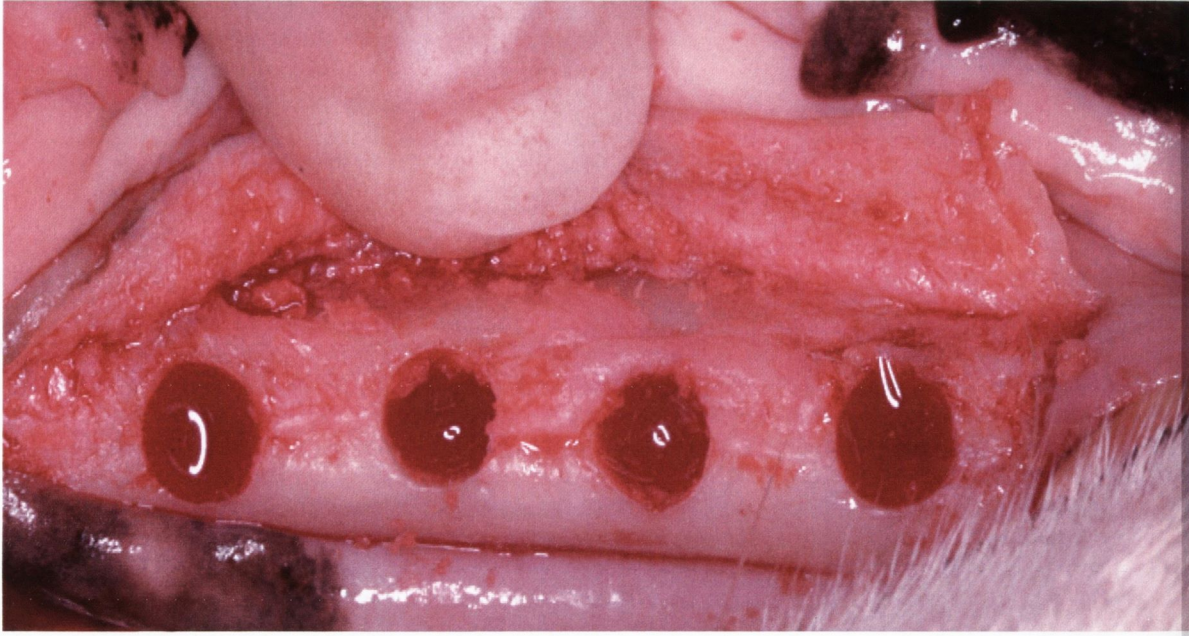


Figure 3.7 Four osteotomy sites were prepared.

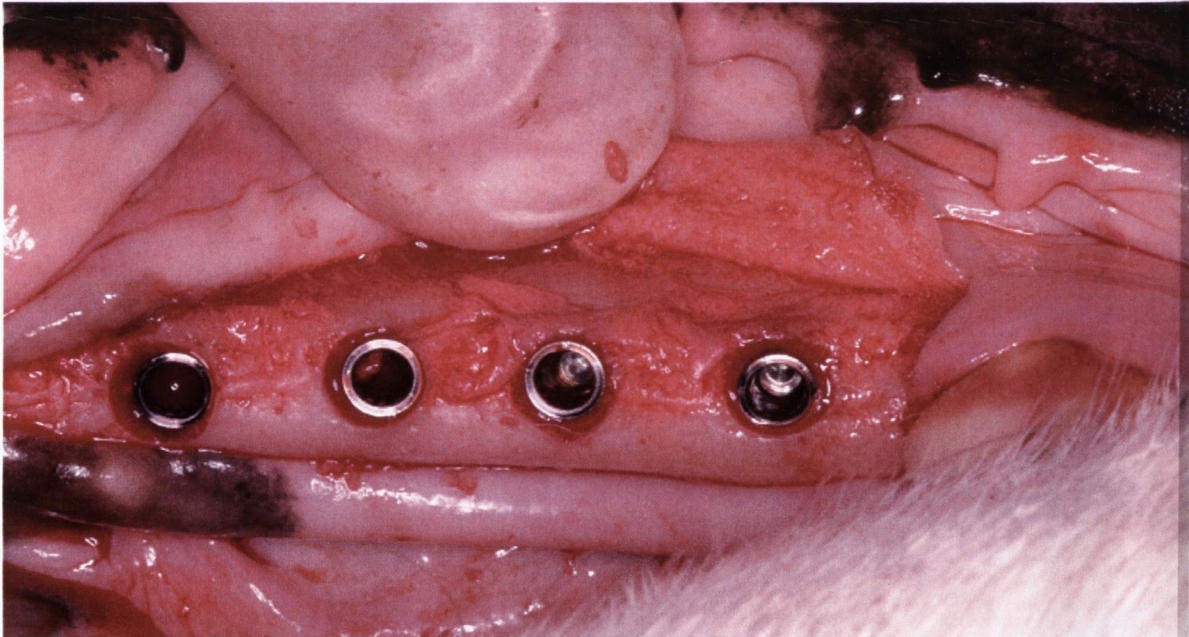


Figure 3.8 Implants were fully inserted in the prepared sites.

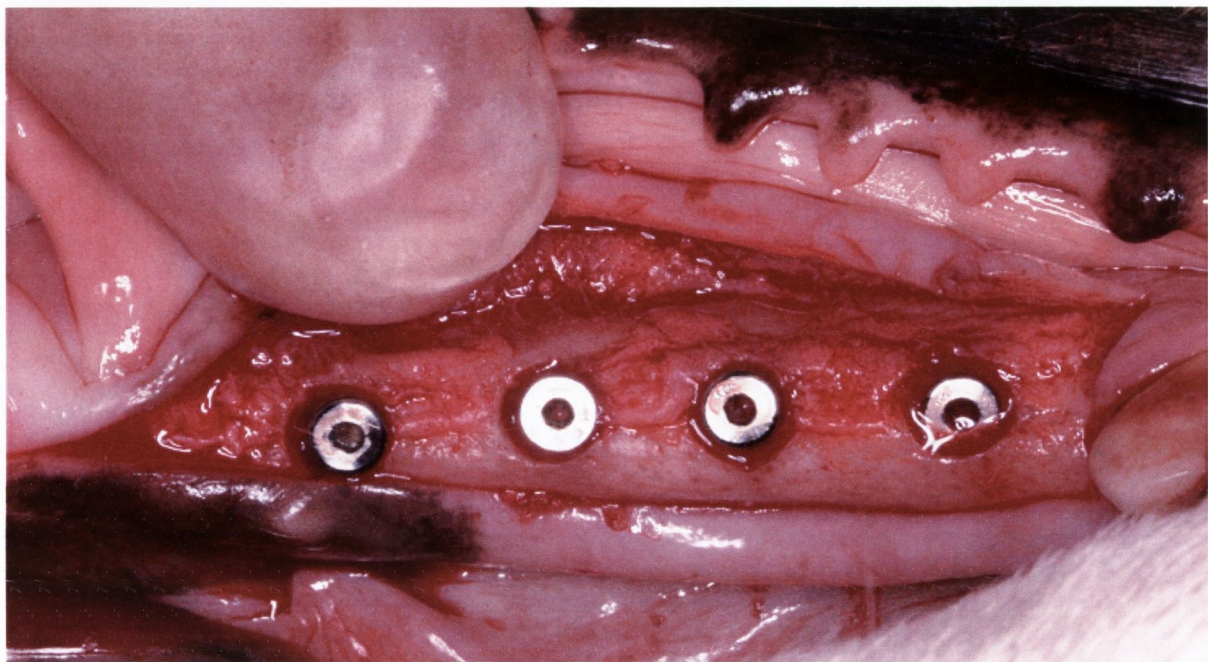


Figure 3.9 Cover screws were placed.

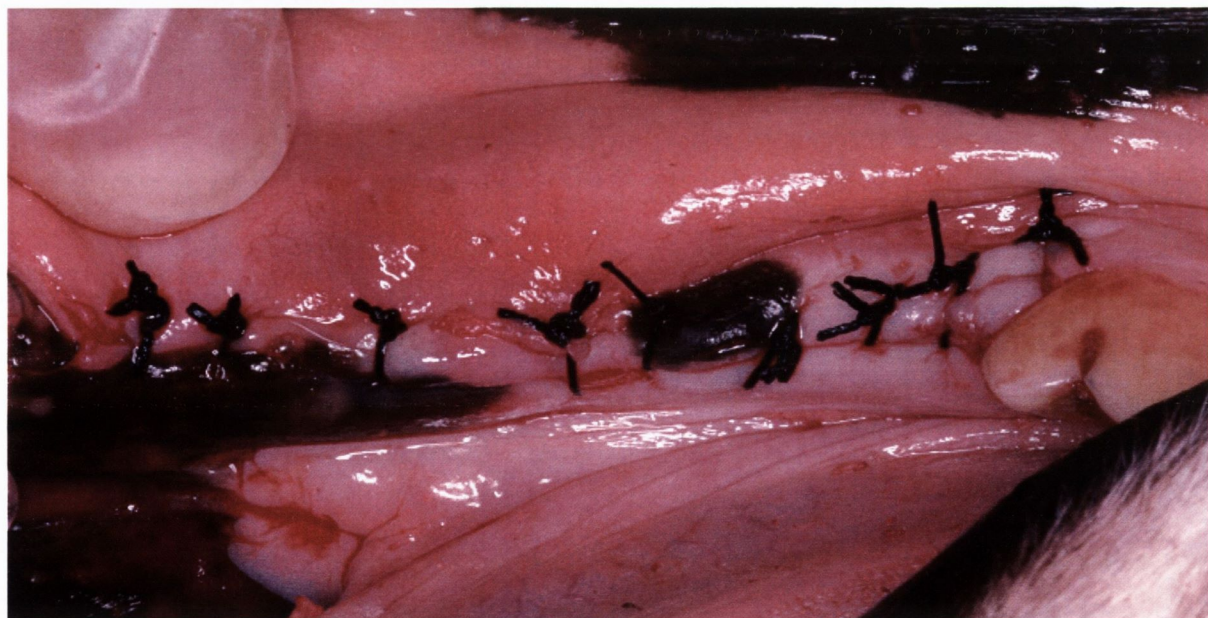


Figure 3.10 Flap sutured back

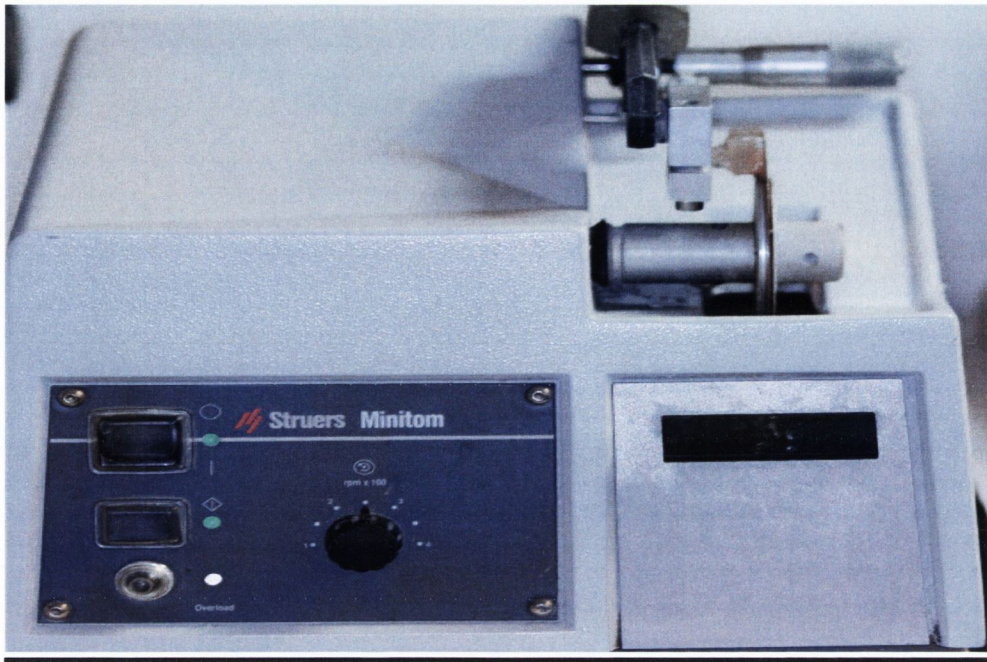


Figure 3.11 Machine that used for sectioning the specimens

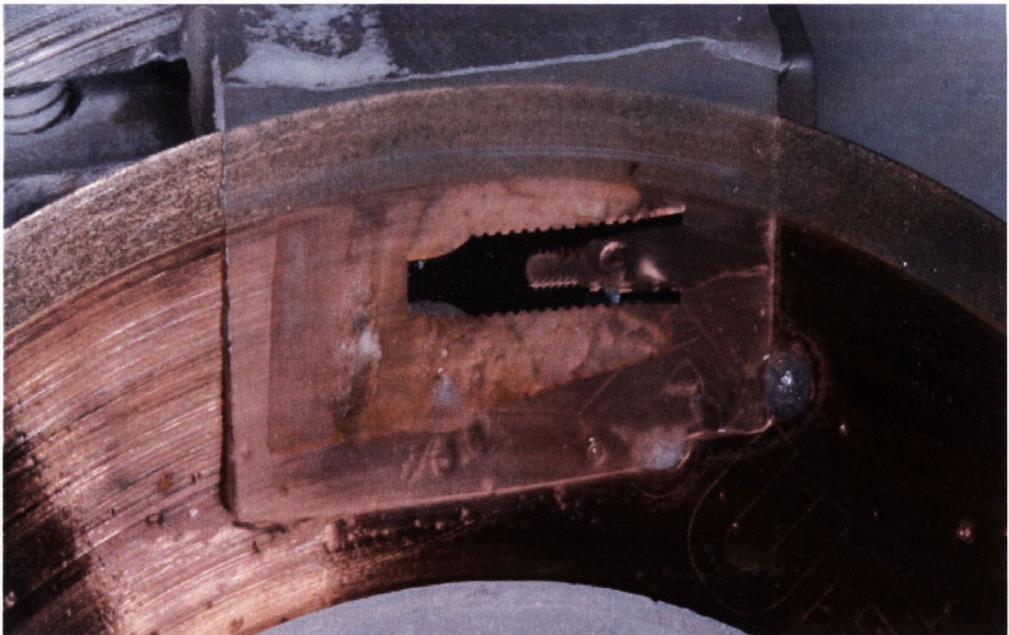


Figure 3.12 Machine during sectioning one specimen

CHAPTER 4

RESULTS

4.1 Analysis at implant level.

Measurement 1 - The linear measurement of bone in direct contact with the implant surface (BIC) by site, type and part.

The model was significant ($F = 4.8433$; $P = 0.0001$) and explained 60% of the variance. The results of the effect test are displayed in table 4.1.

Table 4.1.1 Effect tests for implant site, type and part.

Source	F Ratio	P Value
Implant site	1.5	0.23
Type	1.5	0.23
Part	21.2	0.0001

Only implant part was significant ($P = 0.0001$).

The means and the standard deviations for measurement of percentage of bone in direct contact with the implant surface (BIC) by implant site, type and part are displayed in tables 4.1.2, 4.1.3, 4.1.4 and figures 4.1.1, 4.1.2, 4.1.3.

Table 4.1.2 Means and standard deviations for measurement of percentage of bone in direct contact with the implant surface (BIC) by implant site.

Implant site	Mean	Standard deviation
First premolar	59.3	0.36
Second premolar	74.1	0.24
Third premolar	75	0.21
Fourth premolar	68	0.3

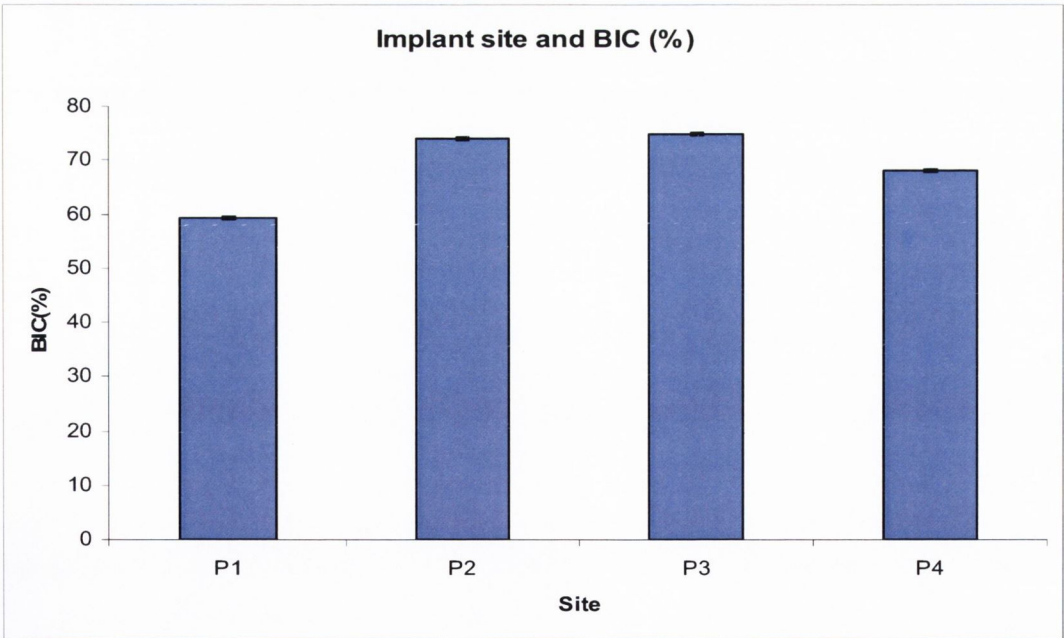
Table 4.1.3 Means and standard deviations for measurement of percentage of bone in direct contact with the implant surface (BIC) by implant type.

Implant type	Mean	Standard deviation
Nanotite™ implant	65.46	0.22
Osseotite® implant	72.7	0.33

Table 4.1.4 Means and standard deviations for measurement of percentage of bone in direct contact with the implant surface (BIC) by implant part.

Implant part	Mean	Standard deviation
Part that was previously contaminated	41.9	0.26
Part that was previously opposed by soft tissues	81.6	0.24
Part that was encased by bone	83.8	0.1

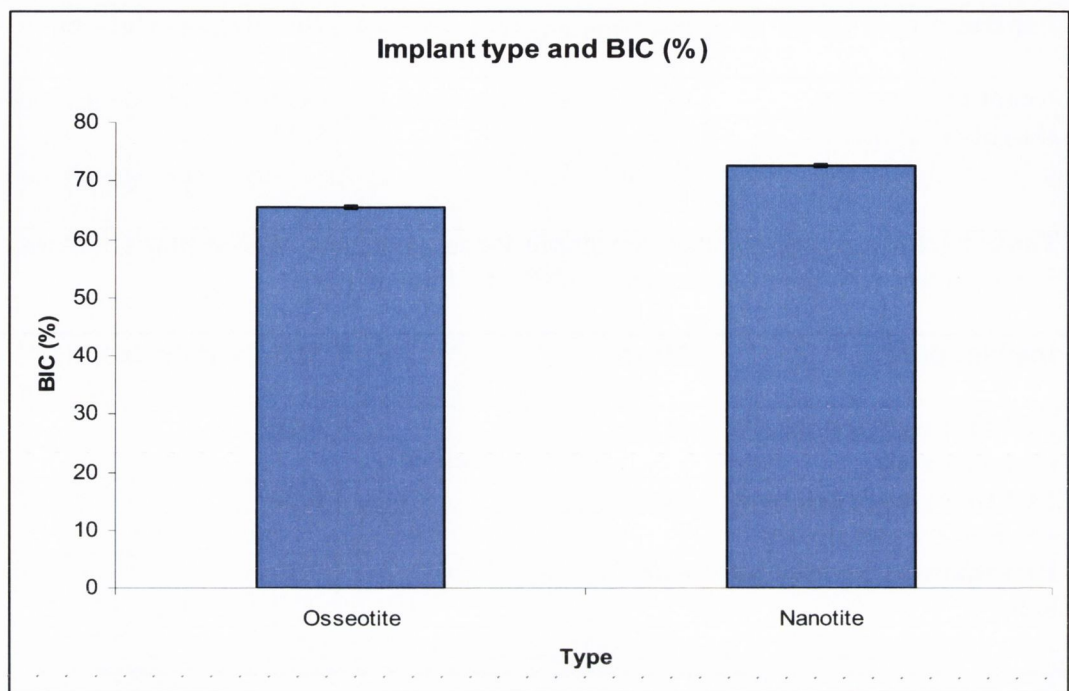
Figure 4.1.1 Means and standard deviations for the percentage of bone in direct contact with the implant surface (BIC) by implant site.



P1= First premolar, p2 = Second premolar, p3 = Third premolar, p4 = Fourth premolar.

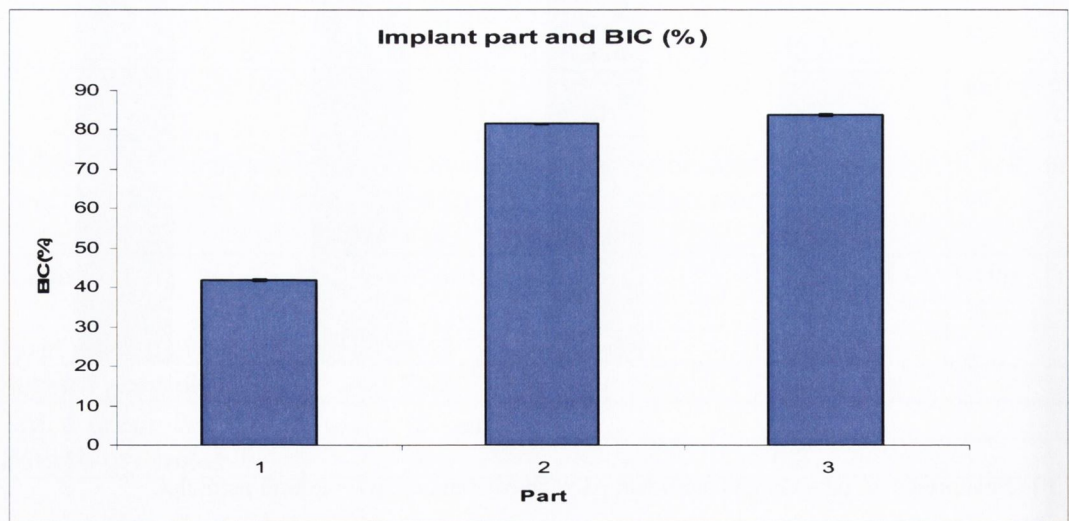
Second and third premolar sites showed higher BIC, nevertheless the differences were not significant (p =0.23).

Figure 4.1.2 Means and standard deviations for the percentage of bone in direct contact with the implant surface (BIC) by implant type.



Nanotite[™] implants showed tendency to have higher BIC than Osseotite[®] implants, nevertheless the difference was not significant ($p = 0.23$).

Figure 4.1.3 Means and standard deviations for the percentage of bone in direct contact with the implant surface (BIC) by implant part.



1 = Part of the implant that was previously contaminated and surrounded by bone defect.

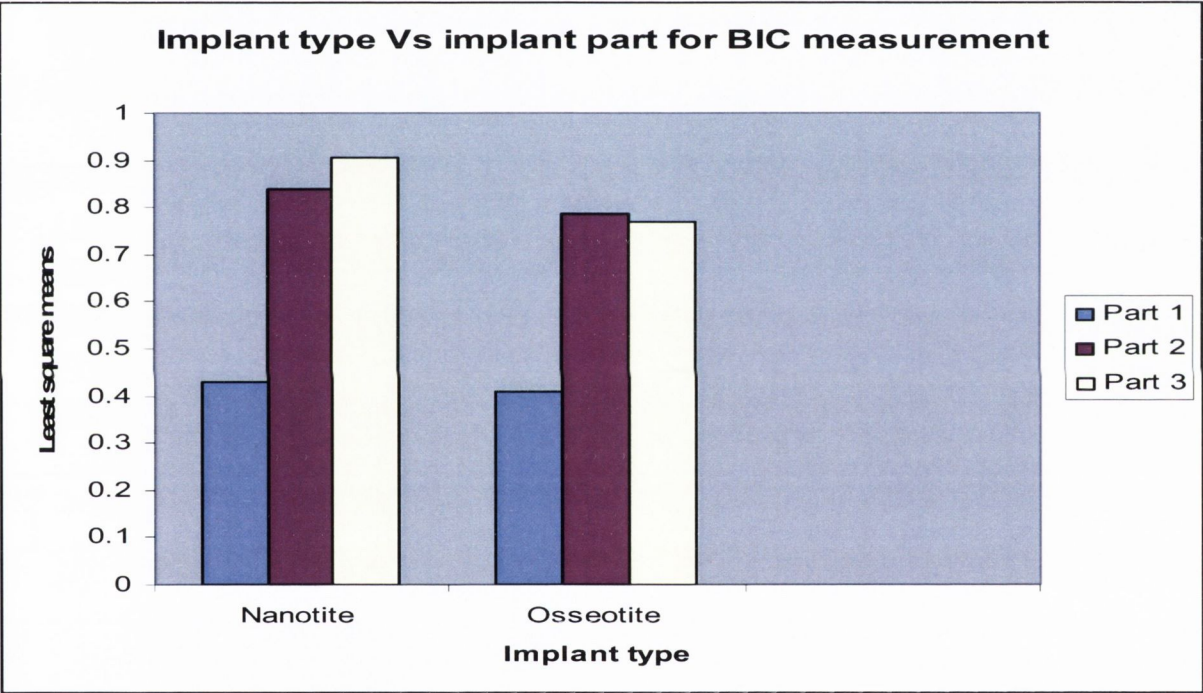
2 = Part of the implant that was previously surrounded by soft tissue alone.

3 = Part of the implant that was encased by bone.

Implant part 1 showed significantly lowest percentage of bone in direct contact with the implant surface ($p = 0.0001$).

There was no significant interaction between implant types and parts ($F = 0.3339$; $P = 0.7184$). The least square means for implant types broken down by implant parts are graphically represented in figure 4.1.4.

Figure 4.1.4 least square means for implant type broken down by implant parts.



Implant types did not influence BIC for implant parts; nevertheless Nanotite™ implants showed the tendency to have higher BIC than Osseotite® implants.

Measurement 2 - Measurement of bone area within the threads by site, type and part.

The model was significant ($F = 3.1788$; $P = 0.0024$) and explained 50% of the variance. The results of the effect test are displayed in table 4.1.5.

Table 4.1.5 Effect tests for implant site, type and part.

Source	F Ratio	P Value
Implant site	1.3	0.31
Implant type	5.114	0.03
Implant part	8.9	0.0007

Implant part and type were significant ($P = 0.0007$ and 0.0299 respectively).

The Means and standard deviations for measurement of percentage of bone area within threads by implant site, type and part are displayed in tables 4.1.6, 4.1.7, 4.1.8 and figures 4.1.5, 4.1.6, 4.1.7.

Table 4.1.6. Means and standard deviations for measurement of percentage of bone area within threads by implant site.

Implant site	Mean	Standard deviation
First premolar	63.8	0.22
Second premolar	71.7	0.25
Third premolar	79	0.27
Fourth premolar	72.8	0.22

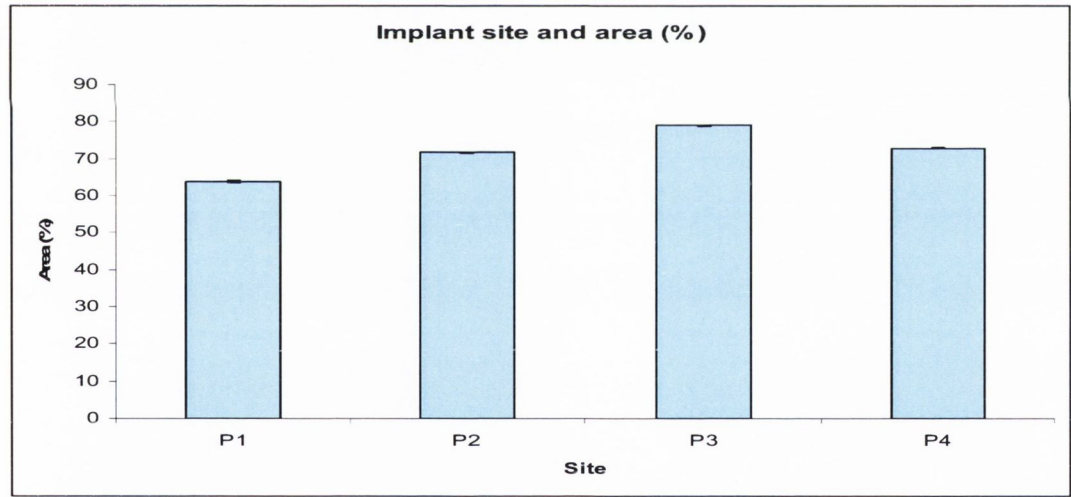
Table 4.1.7. Means and standard deviations for measurement of percentage of bone area within threads by implant type.

Implant type	Mean	Standard deviation
Osseotite [®] implat	65.5	0.21
Nanotite [™] implant	78.13	0.25

Table 4.1.8. Means and standard deviations for measurement of percentage of bone area within threads by implant part.

Implant part	Mean	Standard deviation
Part that previously contaminated	55.3	0.26
Part that previously opposed by soft tissue	80.44	0.2
Part that encased by bone	79.8	0.15

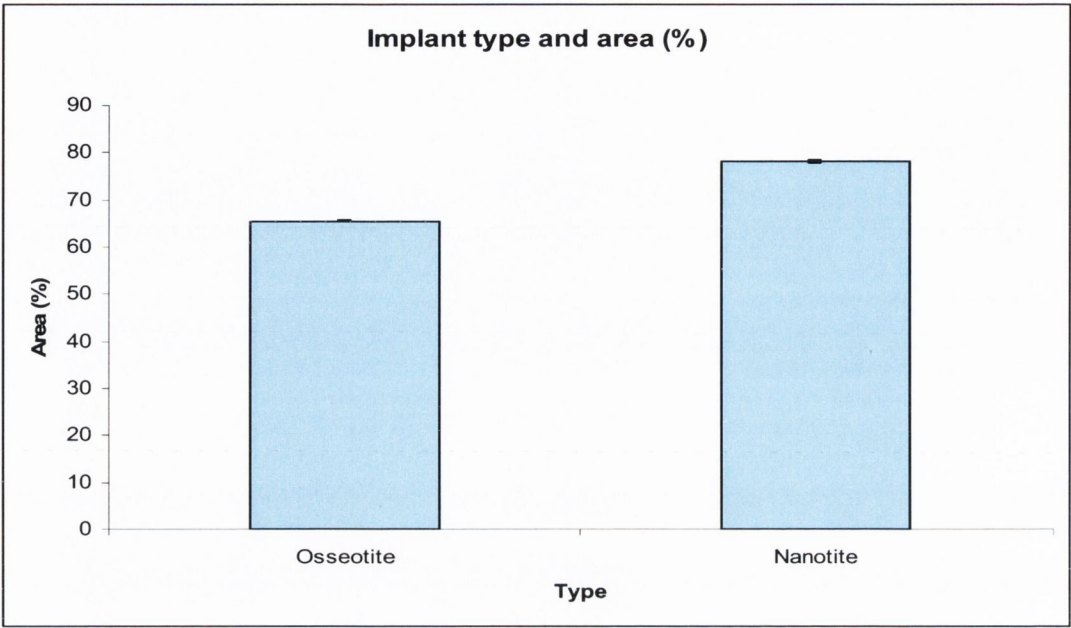
Figure 4.1.5 Means and standard deviations for measurement of percentage of bone area within threads by implant site.



P1= First premolar, p2 = Second premolar, p3 = Third premolar, p4 = Fourth premolar.

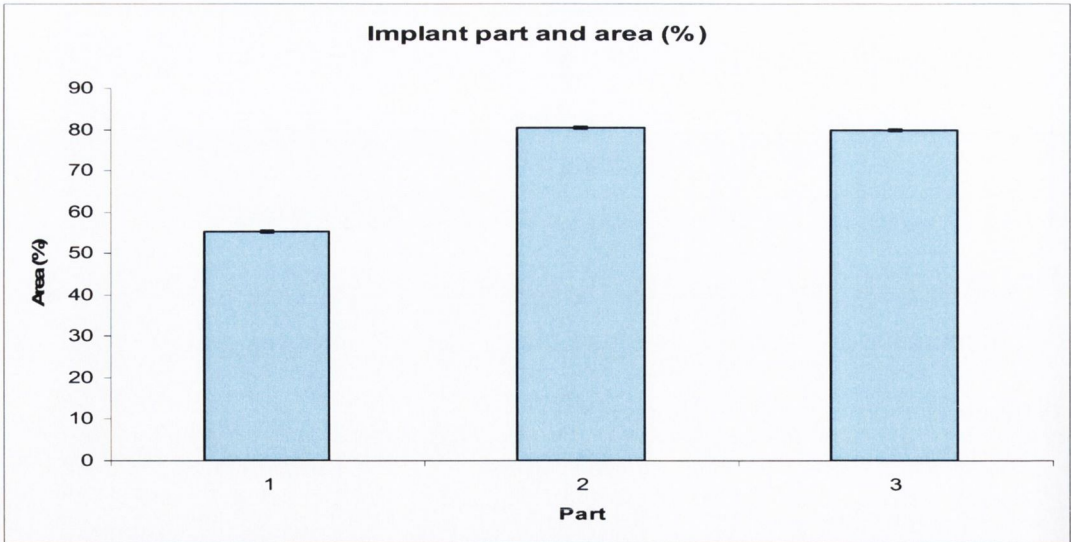
Third premolar sites showed the highest bone area within threads; however the differences were not significant (p =0.31).

Figure 4.1.6 Means and standard deviations for measurement of percentage of bone area within threads by implant type.



Nanotite[™] implants showed significantly higher bone area within threads than Osseotite[®] implants ($p=0.03$).

Figure 4.1.7 Means and standard deviations for measurement of percentage of bone area within threads by implant.

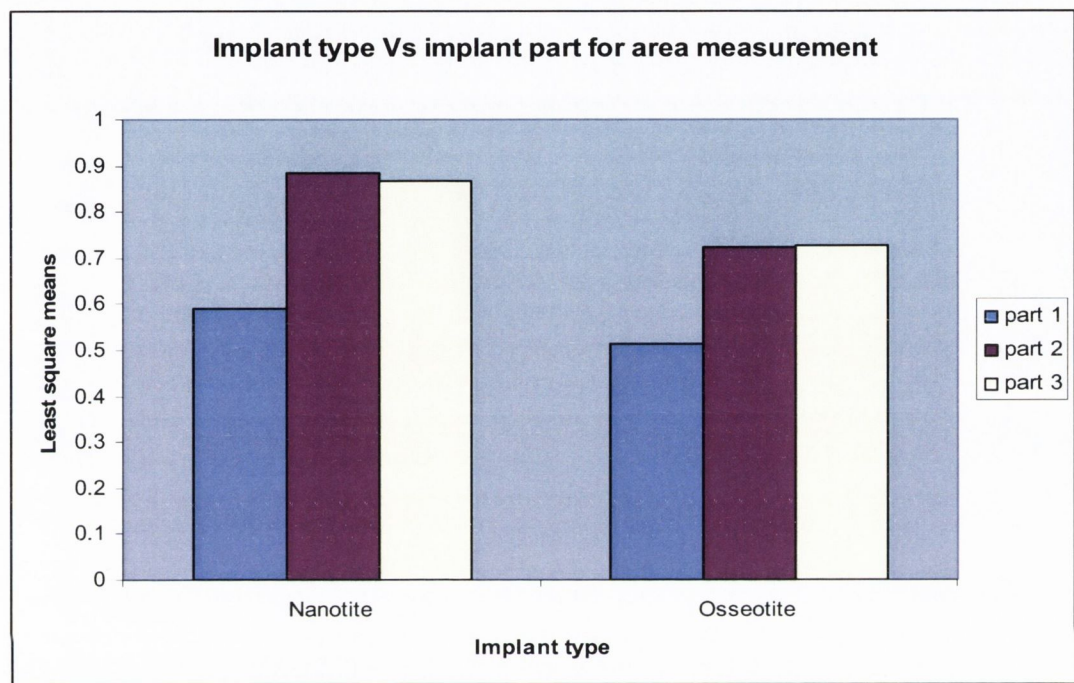


- 1 = Part of the implant that was previously contaminated and surrounded by bone defect.
- 2 = Part of the implant that was previously surrounded by soft tissue alone.
- 3 = Part of the implant that was encased by bone.

Implant part 1 showed significantly lowest percentage of bone area within threads ($p=0.0007$).

There was no significant interaction between type of implant and parts ($F = 0.1804$; $p = 0.8357$). The least square means for type of implant broken down by parts represented in figure 4.1.8.

Figure 4.1.8 least square means for implant type broken down by implant parts.



Implant type did not influence bone area within threads for the implant parts, nevertheless Nanotite™ implants showed tendency to have higher bone area within threads than Osseotite® implants.

4.2 Analysis at dog level

The data was also analysed across the four dogs for the two types of implants. The means and the 95% confidence intervals for the part of implant that was previously contaminated and surrounded by bone defect are displayed in table 4.2.1.

Table 4.2.1 Means and 95% confidence intervals for the two implant types and for the part of implant that was previously contaminated and surrounded by bone defect.

Measurement	Implant type	Mean	95% confidence interval
Linear	1	0.407	0.207; 0.607
	2	0.430	0.22; 0.630
Area	1	0.512	0.309; 0.715
	2	0.592	0.389; 0.795

There was no significant difference between the means of the two implant types for linear measurement (mean difference = 0.022, 95% confidence interval -0.26; 0.305, $p = 0.867$) and for the area measurement (mean difference = 0.08, 95% confidence interval -0.206; 0.336, $p = 0.558$).

The means and the 95% confidence intervals for the part of implant that was previously surrounded by soft tissues alone are displayed in table 4.2.2.

Table 4.2.2 Means and 95% confidence intervals for the two implant types and for the part of implant that was previously surrounded by soft tissues alone.

Measurement	Implant type	Mean	95% confidence interval
Linear	1	0.787	0.602; 0.972
	2	0.843	0.658; 1.02
Area	1	0.725	0.578; 0.871
	2	0.883	0.737; 1.03

There was no significant difference between the means of the two implant types for the linear measurement (mean difference = 0.056, 95% confidence interval -0.205, 0.318, $p = 0.652$) and for the area measurement (mean difference = 0.158, 95% confidence interval -0.048; 0.365, $p = 0.122$).

The means and the 95% confidence intervals for the part of implant that was encased by bone are displayed in table 4.2.3.

Table 4.2.3 Means and 95% confidence intervals for the two implant types and for the part of implant that was encased by bone.

Measurement	Implant type	Mean	95% confidence interval
Linear	1	0.768	0.719; 0.817
	2	0.906	0.857; 0.955
Area	1	0.728	0.627; 0.83
	2	0.867	0.766; 0.968

The Nanotite™ implant showed significantly more bone in direct contact with implant surface (mean difference = 0.137, 95% confidence interval 0.068; 0.206, p = 0.0008) and more bone area within threads than Osseotite® implant (mean difference = 0.138, 95% confidence interval -0.004; 0.282, p = 0.05).

4.3 Histological Observation



Figure 4.3.1

Figure 4.3.1 Mesio-distal section of Nanotite™ implant (dog 1x third premolar)

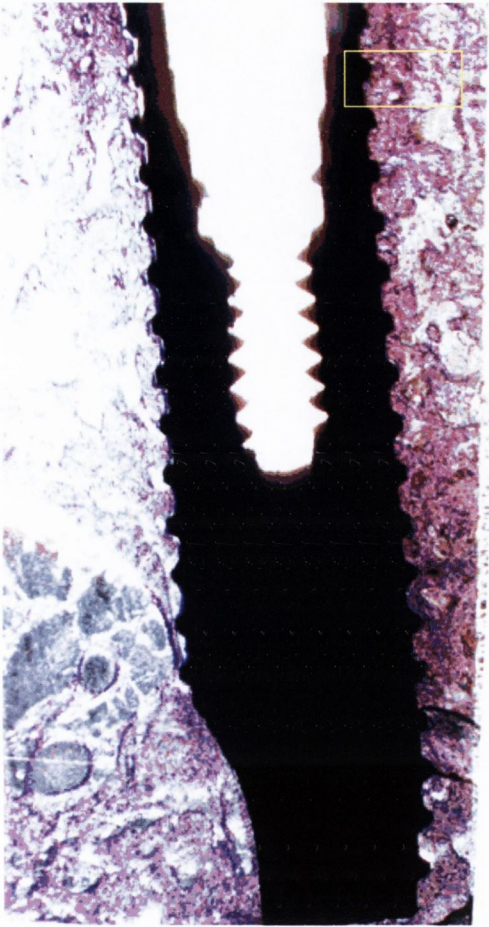


Figure 4.3.2

Figure 4.3.2 Mesio-distal section of Nanotite™ implant (dog 2 x second premolar).

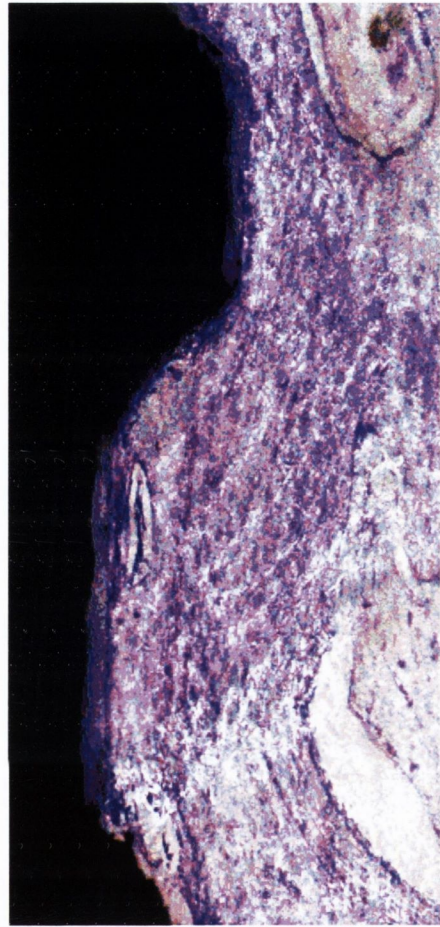


Figure 4.3.3

Figure 4.3.3 higher magnification (x 200) of the implant part that was previously contaminated and surrounded by bone defect (dog 2 x second premolar)



Figure 4.3.4

Figure 4.3.4 Mesio-distal section of Osseotite[®] implant (Dog 2x fourth premolar)

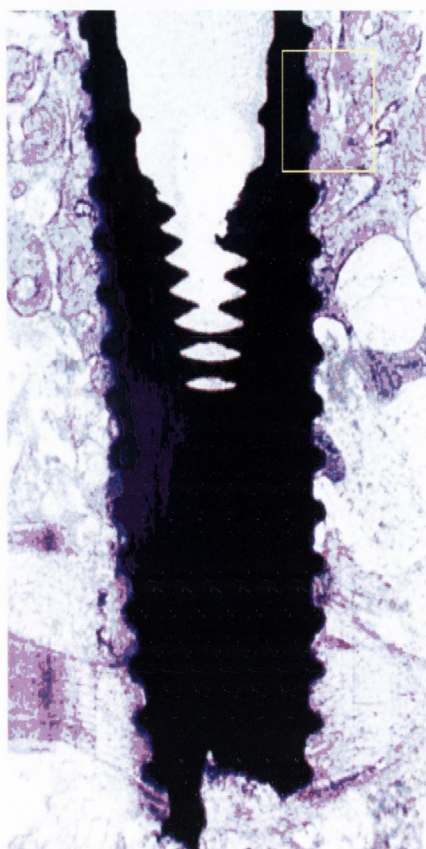


Figure 4.3.5

Figure 4.3.5 Mesio-distal section of Osseotite[®] implant (dog 3 x third premolar).

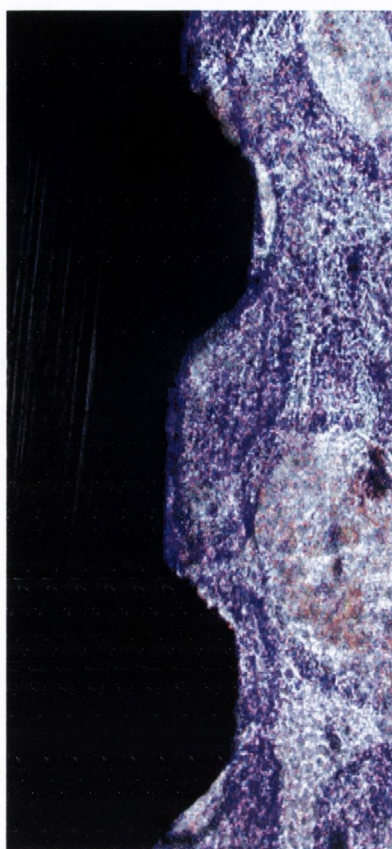


Figure 4.3.6

Figure 4.3.6 higher magnification (x 200) of the implant part that was previously contaminated and surrounded by bone defect (dog 3 x third premolar).

CHAPTER 5

DISCUSSION & CONCLUSIONS

Experiments in animal models have shown that re-osseointegration is possible following treatment of peri-implantitis by different surgical techniques. However, predictability of attainment of significant amount of re-osseointegration seems to be questionable (Singh *et al.* 1993, Wetzel *et al.* 1999, Shibli *et al.* 2003, Schwartz *et al.* 2006b).

Contamination of implant surface was considered to be a factor that would prevent re-osseointegration following treatment of ligature-induced peri-implantitis in animals (Erricson *et al.* 1996, Persson *et al.* 1996, 1999, 2001a). Contrary to this, results from studies using a different model in which plaque accumulation was allowed to occur on implant surfaces exposed to oral cavity indicate the biological possibility of re-osseointegration following decontamination of these implants surfaces when reinserted into defect free healthy sites (Kolonidis *et al.* 2003, Alhag *et al.* 2008).

The primary aim of the present study was to investigate whether osseointegration can occur to implants surfaces that had previously been contaminated with bacterial plaque when these implants were reinserted into healthy sites that are surrounded by bone defects. The second aim was to compare the amount of osseointegration around Osseotite[®] implants (Biomet 3I, Palm Beach Gardens, USA) with that around Nanotite[™] implants (Biomet 3I, Palm Beach Gardens, USA) in beagle dogs.

Results demonstrated that osseointegration can occur to surfaces that were previously contaminated and surrounded by bone defects. Several studies have shown that previously contaminated implants surfaces surrounded by bone defects have the capacity to re-osseointegrate after being decontaminated (Persson *et al.* 2001b,

Hurzeler *et al.* 1997, Schou *et al.* 2003). In these studies peri-implantitis was induced by sub-gingival placement of cotton ligature around the dental implants. The present study utilized a different model where contamination of the implant surface was achieved by exposing the coronal 5 mm of each implant to the oral environment for five weeks and the bone defect was produced mechanically by the drill during preparation of the osteotomy sites. It must be considered that this model may be associated with different configuration of the bone defect around the implant and that different microbial species may populate the implant surface compared to ligature induced peri-implantitis.

In the present study, although re-osseointegration did occur, the percentage of bone in direct contact with the implant surface (BIC) for the part that was previously contaminated and surrounded by bone defect (41%) was significantly lower than for the part that was previously opposed by soft tissue alone (81%) and the part that was encased by bone (83%). One possible explanation for this may be the tendency of implants to lose bone at the coronal alveolar margin during healing and remodeling stage (Alhag *et al.* 2008). Previous contamination may also explain lower osseointegration around the part that was previously contaminated and surrounded by bone defect. In the present study some of the implants were placed at an angle which resulted in a bone defect of more than 1 mm around the coronal 5 mm of the implant. As studies have shown that osseointegration will be influenced by the dimension of the bone defect around the implant (Polyzois *et al.* 2007), this may also explain why the part that was previously contaminated and surrounded by bone defect had lower osseointegration.

Site of the implant was not found to influence either the percentage of bone in direct contact with the implant surface (BIC) or percentage of bone area within the threads. This finding is in agreement with Polyzois *et al* (2007) and Alhag *et al.* (2008). However; it is at variance with study by Kolonidis *et al.* (2003). These authors investigated the effect of contamination on osseointegration of dental implants in labrador dogs. They used machined surface Nobel Biocare implants previously contaminated and placed into defect free healthy sites. Results showed that second premolar sites tended to display more osseointegration and bone area within the threads than the other sites.

There was no significant difference of percentage of bone in direct contact with the implant surface (BIC) between Osseotite[®] implants (Biomet 3I, Palm Beach Gardens, USA) and Nanotite[™] implants (Biomet 3I, Palm Beach Gardens, USA). However, the percentage of bone area within threads was found to be significantly higher in Nanotite[™] implants than Osseotite[®] implants. In contrast to the result of the present study, Orisini *et al.* (2007) reported significantly greater osseointegration (BIC) of Nanotite[™] implants than Osseotite[®] implants. These authors used sterile implants that were placed into defect free healthy sites in human volunteers.

When data analyzed across the four dogs for the two types of implants, Nanotite[™] implant showed significantly more bone in direct contact with implant surface and more bone area within threads than Osseotite[®] implant for the part of the implant that was encased by bone.

When interpreting the results of the present study consideration should be given to some limitations. First, dogs may not respond in a similar way as humans, making

direct application of the results to humans inappropriate. Second, the small number of animals used which leads to a certain amount of uncertainty from statistical standpoint.

Conclusions

In conclusion, the present study in dogs has demonstrated the biological possibility of re-osseointegration of previously contaminated implant surfaces surrounded by bone defects. This would suggest that further studies in the future are needed to establish other potentially limiting factors with regard to re-osseointegration such as clot stability/adhesion and cell differentiation and migration. The present study also demonstrated that Nanotite[™] implant showed significantly greater bone area within thread than Osseotite[®] implants. This may suggest further investigations are needed to ascertain the importance of Nanotite[™] implant surface.

CHAPTER 6

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CHAPTER 7

APPENDICIES

APPENDIX 1 The complete data set of the measurements

Dog	Implant site	type	Part	Linear measurement	Area
1	1	1	1	0.15	0.9
1	1	1	2	0.87	0.74
1	1	1	3	0.67	0.68
1	2	1	1	0.35	0.48
1	2	1	2	0.76	0.74
1	2	1	3	0.84	0.73
1	3	2	1	0.75	0.92
1	3	2	2	0.94	0.99
1	3	2	3	0.93	0.98
1	4	2	1	0.03	0.35
1	4	2	2	0.89	0.93
1	4	2	3	0.76	0.97
2	1	1	1	0.25	0.31
2	1	1	2	0.83	0.77
2	1	1	3	0.77	0.62
2	2	2	1	0.68	0.74
2	2	2	2	0.98	0.92
2	2	2	3	0.82	0.73
2	3	2	1	0.41	0.41
2	3	2	2	0.91	0.92
2	3	2	3	0.93	0.99
2	4	1	1	0.63	0.75

2	4	1	2	0.73	0.64
2	4	1	3	0.75	0.69
3	1	2	1	0.51	0.75
3	1	2	2	1	0.86
3	1	2	3	0.93	0.83
3	2	2	1	0.81	0.94
3	2	2	2	1	1
3	2	2	3	0.92	0.98
3	3	1	1	0.41	0.24
3	3	1	2	0.6	1
3	3	1	3	0.77	0.78
3	4	1	1	0.72	0.74
3	4	1	2	0.76	0.58
3	4	1	3	0.78	0.75
4	1	2	1	0.13	0.24
4	1	2	2	0.04	0.47
4	1	2	3	0.96	0.49
4	2	1	1	0.19	0.21
4	2	1	2	0.77	0.35
4	2	1	3	0.77	0.78
4	3	1	1	0.56	0.47
4	3	1	2	0.98	0.98
4	3	1	3	0.8	0.8
4	4	2	1	0.12	0.39
4	4	2	2	0.99	0.98

4 4 2 3 1 0.97

Implant site:

1. Lower first premolar
2. Lower second premolar
3. Lower third premolar
4. Lower fourth premolar

Implant type:

1. Osseotite[®]
 2. Nanotite[™]
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Implant part:

1. Previously completely exposed
2. Opposed by soft tissue
3. opposed by bone