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Comparison of piezoimplants versus conventional cylindrical implants in minipigs: stability analysis

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THESIS

COMPARISON OF PIEZOIMPLANTS VERSUS CONVENTIONAL CYLINDRICAL IMPLANTS IN MINIPIGS: STABILITY ANALYSIS.

by

LORENA MARIE CORZO

D.M.D., Central University of Venezuela (Caracas, Venezuela 2009)

AEGD, University of Florida (2015)

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READERS APPROVAL

First Reader: ________________________.

Wayne Gonnerman

Assistant Professor of Periodontology

Second Reader: __________________________________.

Serge Dibart DMD

Chair of Periodontology, Director of the Advanced Specialty Education

Program in Periodontics at GSDM, Professor, Department of Periodontology.

DEPARTMENT APPROVAL

Department Chairman

Serge Dibart, D.M.D.

Director, Professor and Chairman, Department of Periodontology and Oral Biology

Boston University, Henry M. Goldman School of Dental Medicine

____

Signature **Date**

DEDICATION

To my parents, I would like to thank for their continuous support, and understanding during my life. On the other side of the continent but, always encouraged me to continue my studies. Thank you for your unconditional love!

To my love Mourad, I would like to thank you for your understanding through the long working days and weekends. You have been my best friend through rough times far from home.

COMPARISON OF A NON-CYLINDRICAL PROTOTYPE IMPLANT (PIEZOIMPLANT) VERSUS CONVENTIONAL CYLINDRICAL IMPLANT IN MINIPIGS. PART I: STABILITY ANALYSIS.

LORENA CORZO

Boston University Henry M. Goldman School of Dental Medicine, Boston, MA

Department of Periodontology.

*Department of Biomedical Engineering. Fraunhofer USA - Center for Manufacturing Innovation.

Advisors: Xu, Wanpeng; Sauer-Budge, Alexis*; Dibart, Serge.

ABSTRACT

Aim: To compare the stability of a non-cylindrical implant using piezoelectric drilling (Piezoimplant) with a cylindrical implant with conventional drilling (Nobel Biocare™).

Materials and Methods: Three adult female Gottingen miniature pigs were used for the surgical implantation. Three implants on each quadrant, randomized split-mouth design using cylindrical or non-cylindrical implants (n=36). Osteotomies were prepared using either conventional drilling technique as per manufacturer's instruction (Nobel Biocare™) or using piezoelectric drilling (Piezotome® (P2) (Satelec Acteon, Merignac, France) with new implant prototype tips (Fraunhofer Center for Manufacturing Innovation). The minipigs were sacrificed at 4, 8 and 12-weeks. Stability tests (three per implant) using wireless Periotest® "M" were done at the start point and after euthanization.

Results: R-square (ANOVA) test was plotted comparing implant design, weeks 4, 8 and 12, and location (mandible and maxilla) for stability analysis. In this model, the R-square is only 0.51, which indicates only 51% of the response variability can be explained by the fitted model. Among all the 3 factors, group (experiment vs control) is the most significant one, followed by week. Location significance is the least among the three factors.

Conclusion: In mandibular and maxillary sites in minipigs where non-cylindrical prototype implants (piezoimplant) were inserted by piezoelectric site preparation, statistically significant differences were found between control and test group stability measurements, but no differences in week (4, 8 or 12) and location among the two groups (mandible and maxilla). Stability was like the cylindrical implants. Meaning that Piezoimplants could be an alternative for narrow residual ridges.

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1. INTRODUCTION

Tooth loss due to disease or trauma is still very common. For functional and esthetic reasons over the course of human history there have been numerous and varied attempts to provide effective replacement.

1.1 Population with Total or Partial Edentulism.

About 70% of the United States' population was missing at least 1 tooth and 30% of the population between 50 and 59 years of age showed one or multiple edentulous spaces which increased total edentulism seen in younger adults, by the age of 75 the percentage observed was up to 44% of the cases. These statistics primarily affects an aging and economically disadvantaged vulnerable population(Misch, 2008). The American College of Prosthodontics(ACP), acknowledges that more than 35 million Americans have no teeth, and that 178 million in the U.S. are missing at least one tooth. In the 1940s, edentulism was believed to be inevitable due to aging. Later preventive dentistry was introduced in the 20th century, and more people realized that natural teeth could be retained and saved. The ACP estimates that in the next 15 years, the partially edentulous patient population will increase to more than 200 million individuals, with clear repercussions for the patients' health. Thus, losing a tooth remains a significant concern.

1.2 Morphological changes after tooth loss.

Tooth extraction is one of the most common dental procedures, it could be due to advanced caries, periodontal disease, trauma, fracture, pathology and endodontic lesions. The alveolar healing will be a combination of bone growth and resorption of the alveolar ridge. The most common bone loss is in the horizontal dimension mainly of the buccal aspect. Vertical dimension loss, can also occur resulting in a narrower and shorter ridge. (Van der Weijden, Dell'Acqua, & Slot, 2009).

Residual Ridge is a term used to described the clinical aspect of the alveolar ridge after healing of bone and soft tissues after tooth loss. The socket will be progressively filled with bone. One of the features after extraction is that the residual ridge bone will undergo a slow life-long catabolic remodeling, resulting in a large amount of bone reduction. (Jahangiri, Devlin, Ting, & Nishimura, 1998).

Resorption of the alveolar ridge following tooth loss has become a significant problem specially if is in the anterior area. The dental team faces the challenge of creating prosthetic restorations to restore not only function but, also an harmonious blend with the adjacent natural dentition. (Van der Weijden et al., 2009).

1.3 Classification of Edentulous Maxilla and Mandible.

Cawood and Howell conducted a randomized cross-sectional study of 300 dried skulls (Cawood & Howell, 1988). Changes in the dimension of the basilar process were not significant regardless of the degree of atrophy of the alveolar process. Based on this study diagrams showing the most commonly observed changes in the alveolar process of the mandible and a descriptive classification of these changes were developed.

Class I - dentate.

Class II -immediately post extraction.

Class III- well-rounded ridge form, adequate in height and width.

Class IV - knife-edge ridge form, adequate in height and inadequate in width.

Class V flat ridge form, inadequate in height and width.

Class VI - depressed ridge form, with some basalar loss evident.

1.4 Bone quality classification.

Alveolar bone is composed of cells and mineralized extracellular matrix, its remodeling/resorption response under action of mechanical loads is the ultimate predictor of implant longevity (Bidez & Misch, 1992). From a macroscopic perspective, bone can be described as either dense to porous compact (cortical) bone or coarse to fine trabecular (cancellous) bone. On a microstructural perspective, the trabeculae of cancellous bone consist of parallel lamellae; cortical bone presents circumferentially arranged parallel lamellae called osteons that composed the Haversian system (Bidez & Misch, 1992).

Based on the radiographic appearance and the resistance at drilling, bone quality has been categorized in four groups:

Type 1: homogenous compact bone.

Type 2: thick layer of compact bone surrounding a core of dense trabecular bone.

Type 3: thin layer of cortical bone surrounding a core of dense trabecular bone.

Type 4: thin layer of cortical bone surrounding a core of low density trabecular bone of poor strength. These differences can be associated with different anatomical areas in the mandible or maxilla. Bone quality is significant when considering an implant placement site and is a factor for implant success (Brånemark, 1986).

1.5 Implant History.

Over centuries losing teeth has been a concern for human beings. As the lifespan of the population increased tooth loss also increased. Diverse cultures have used different methods to treat oral diseases, maintain and restore teeth. These ancient dental practitioners included Hindus, Chinese, Japanese, Phoenicians, Hebrews, Greeks, Romans, Etruscans, and Mayans, treated edentulous spaces with extracted teeth from slaves and they used metals and precious gems for cosmetic enhancement. The first registered implant placement was done in the Mayan civilization in the 7th century AD, and was constructed of sea shells and placed in the mandible (Garg, 2010). The main objective in modern dentistry is to rehabilitate the patient's normal contour, function, comfort, esthetics, speech, and health, whether by removing caries from a tooth or replacing several teeth (Misch, 2008). Since the beginning of the 20th century, dentists have experimented with insertion of metals in the alveolar bone to allow permanent tooth placement. In the 30s experiments using vitallium orthopedic screw fixtures in dogs and human subjects were used to restore individual teeth. After World Word II, Dr. Goldberg, a former soldier, having seen metal used in other parts of the body proposed their use in the mouth and in association with Dr. Gershkoff produced the first successful sub-periosteal implant. Different implant designs and materials have been used over the years, including those by Seger-Dorez, Lehman, Pretto, and Ted Lee used spiral cylindrical devices that allowed blood flow and bone growth (Brånemark, 1986). Dr. Linkow designed a flat vent-plant implant, that differed from the spiral design (Linkow, 1969). It was manufactured in various configurations to accommodate the type of bone ridge and the area requiring restored dentition. During the 50s, 60s and 70s different subperiosteal and transosseous mandibular devices were used. In the 1960's Dr. Brånemark, an orthopedic surgeon, after years of investigations using rabbit femurs, observed that the titanium-hollow cylinders used to investigate the blood flow in live animas firmly attached to the femurs; this observation gave rise to the concept that he later called "Osseointegration", a state in which the bone integrates with the titanium implant without soft tissue intervention (Meffert, 1986). Dr. Brånemark proposed using titanium in the rehabilitation of edentulous jaws. In the 70's and 80's Schroeder and Straumann in Switzerland also developed an implant system (Gaviria, Salcido, Guda, & Ong, 2014). In 1982, the Food and Drug Administration approved the use of titanium dental implants and in 1983, Dr. Matts Andersson developed what became Nobel Biocare in Zurich, Switzerland.

Implants have become an essential treatment option in modern dentistry, offering a wide range of benefits, including improved function, increased patient comfort, a means to replace partially edentulous dentition and the maintenance of proprioceptive feedback following the loss of a tooth. (Baker et al., 2012)

1.5.1 Types of dental implants

The most frequently used dental implants are endosseous, typically designed in a screw-shape form. Dental implants are now made of pure grade 4 Titanium which is resistant to corrosion and stronger than other grades. However, there are others that use titanium alloys like Ti6A14V that show more resistance to fatigue.(Gaviria et al., 2014)

Implants differ by the overall shape, cylindrical or conical, and thread topography. Other factors will contribute to the survival of the implant such as body shape, size, chemical surface treatment and composition and topographical features.

(Gaviria et al., 2014) Improved design of dental implants will ensure primary stability by allowing strong bone-implant interface due to osseointegration. Different designs are available but they could be group in three categories:

1) Screw threads

2) Solid body press-fit

3) Porous-coated design

Each of them will affect the bone-implant interface due to the different biomechanical properties. Different implant shapes will allow equal distribution of forces and will promote bone growth. Brånemark hypothesized that optimum load transfer from the implant to the osseous tissue should result in no movement (Brånemark, 1986). Improvements in the bioengineering, implant surface design and geometry will increase tissue preservation and faster osseointegration. (da Silva Neto, Joly, & Gehrke, 2014).

1.5.2 Implant surface treatment

As described before dental implants are commonly made from titanium and titanium alloys, due to their strong passivating oxide layer leading to good biocompability, resistance to corrosion and good mechanical properties, during the years different attempts have been made to modify and improve the characteristics of the surfaces that could enhance the stability in the short-term and reduce failures, in order to produce a faster and more physiological integration. (Raphel et al., 2016). Numerous surface modification methods can be found in the market, there are adding methods (titanium plasma and hydroxyapatite) and there are removing methods (microparticle blasting and acid etching) each of them with purpose of obtain macro and micro-roughness characteristics that have showed to improve mechanical stability and osseointegration where cortical bone is inadequate compared to machine or untreated surfaces (Lee, Kim, & Lim, 2011).

The surface topography is one of the principal factors for primary fixation due to the design and features of the implant. This characteristics will control cell recruitment, adhesion, orientation and even gene expression (Ferraris, Bobbio, Miola, & Spriano, 2015).

1.6 Implant Stability

Different success criteria have been used in the implantology field. "Osseointegration is defined as a direct structural and functional connection between living bone and the surface of a functionally loaded implant" (Canullo, Penarrocha, Penarrocha, Rocio, & Penarrocha-Diago, 2014).

Osseointegrated implants, designed for intraoral prosthetic rehabilitation have showed high success rates if certain preconditions are reached. Stability plays a critical role for the long-term clinical success, depending on the quality and quantity of bone, the implant design, and the surgical technique employed. (da Silva Neto et al., 2014; Sennerby & Meredith, 2008)

Implant stability is generally defined as "a measure of the difficulty of displacing an object or system from equilibrium" (Friberg, Sennerby, Linden, Gröndahl, & Lekholm, 1999). It has also been defined as "the capacity of the implant body to withstand loading forces in axial, lateral and rotational directions" (Saha, Pal, & Albright, 1982). Osseointegration increases the stiffness of the bone against the implant surface, creating macro and micro-interlocking, which prevents micro-movements and the formation of fibrous tissue onto the bone-implant connection (Sennerby & Meredith, 2008).

Primary stability results from a mechanical phenomenon in which there is an engagement between the fixture and the bone walls surrounding the implant. This could depended on the local bone quality and quantity, height of the crestal cortical bone, geometry of the implant, and the placement technique used (Sagheb et al., 2017).

After insertion, it is required to have no movement or a biometric stability immediately after placement. This stability will be subject to bone remodeling (Verdonck et al., 2008). Additionally, poor primary stability could be considered a major cause of failure (Roos et al., 1997). Secondary stability is defined as the progressive stability achieved through new bone formation and remodeling during the healing process, which will be affected by bone morphology including the trabecular pattern, density, and the degree of maturation. (Brunski, 1992; Canullo et al., 2014; da Silva Neto et al., 2014; Meredith, 1998). It was also suggested that individual stability testing with radiographic examination should be used when a new implant system is tried. Good documentation is of paramount for the future success of the implant system.

Implant stability must be maintained for the entire healing period to avoid fibrous tissue formation. It is essential for formation and maturation, and to allow ideal stress distribution from mastication. In general, 3-6 months undisturbed healing is prerequisite for achieving bone apposition (Verdonck et al., 2008). Quantitative measurements can predict the best healing period for an implant, good surgical technique, implant dimensions (length and diameter) and surface characteristics. Two major factor that contribute to primary stability are the bone-implant contact and the compressive stresses at the implant-tissue interface.

Different measurements techniques are available, classified as destructive and nondestructive methods. Histomorphologic, tensional test, push-out/pull-out test and removal torque as destructive methods. Percussion test (Periotest), radiography, cutting torque test at placement and resonance frequency analysis (RFA) as nondestructive or noninvasive methods (Meredith 1998).

Periotest® (Seimens, AG, Bensheim, Germany) “electronic instrument designed with a translational hammer mounted inside a handheld probe which is fired by an electromagnet against an accelerometer that impacts the tip against a dental implant”(Meredith, 1998). The tip of the rod has a sensor that quantifies the mobility, by measuring the reaction of the peri-implant tissue to the impact load. The rod after activation impacts the implant 16 times in four second. The rod decelerates when it touches the implant surface and accelerates after rebounds off the surface. The machine measures the damping capacity of natural teeth and implants. More implant stability will be registered if the elapse time is shorter. (Dario, Cucchiaro, & Deluzio, 2002)

Periotest values are recorded through a software that converts the percussions into electronic values; these values are marked from -8(low mobility) to +50(high mobility). As described in the company web side, the Periotest instrument can be used to assess the osseointegration of implants, in table 1 the ranges and the interpretation for the digital values.

Table 1 Assessment of the Osseointegration of the implant

Periotest Value Range	Interpretation
-8 to 0	Good osseointegration, implant is well integrated and pressure can be applied to it.
+1 to +9	A clinical examination is required: the application of pressure on the implant is generally not (yet) possible.
+10 to m+50	Osseointegration is insufficient and no pressure may be allowed to act on the implant.

Table 1. Assessment of the Osseointegration. “Periotest user’s manual” http://www.med-gulden.com/periotest.php. Last modified 8/01/2017

The machine can measure all surfaces of the abutments or prosthesis. However, the rod must make contact at a correct angle and distance. This test is not easy to perform. If the perpendicular contact is more than 20 degrees, or the parallel contact more than 4 degrees, the measurement will be invalid. The rod tip and the surface must maintain 0.6 to 2.0mm distance, and if is bigger than 5mm, the measured values will be insignificant (Schulte, 1988). Only a buccal-lingual mobility can be assessed with this method. Additionally, the sensitivity of this method is not sufficient, it cannot detect minor changes in the implant-tissue surface interface (Park, Lee, Kim, & Lee, 2011).

Another method available called Resonance Frequency Analysis (RFA) was introduced by Meredith in 1998. By connecting an adapter to an implant, the machine uses a frequency of audible range of vibration onto an implant, the stronger the bone-implant contact, the higher the frequency. Ostell™ the commercial brand, used resonance frequencies between 3.5 Khz and 8.5 KHz from a magnetic field is converted in stability quotient, from 1 to 100, where 100 is the highest implant stability. Each implant system requires the respective transducer and magnetic peg which could be a disadvantage when a prototype implant needs to be assessed. (Park et al., 2011).

1.7 Surgical techniques

Osteotomy is one of the most demanding procedures and is related to the success and treatment outcome in implantology (de Ávila Kfouri et al., 2014). Conventional techniques of preparations (cutting drills) have been used to prepared the areas for implant insertion. Even though these instruments are efficient, affordable, relative simple to use and long track record of clinical success (Sagheb et al., 2017), they can have some disadvantages that may cause damage to the tissues, including the generation of debris and bone chips, hematomas, heat production, created mainly due to the high pressure manual movement and the speed required. Additionally, it produces difficulties in maintain geometrical accuracy, and vibration which could difficult an efficient cut (de Ávila Kfouri et al., 2014).

Dentoalveolar ridge management represent a challenge for the successful placement of implants. Horizontal and vertical atrophy of the alveolar ridge are usually present in severe edentulism (class IV to VI Cawood and Howell classification). Several techniques could be considered for alveolar ridge augmentation to allow adequate restorative driven implant position (González-García, Monje, & Moreno, 2011).

Ridge augmentation will include Bone grafting, guided bone regeneration or distraction osteogenesis previous to plan an implant insertion. Specific disadvantages have been reported such as, resorption, limited bone gain, damage of adjacent vital structures (teeth, sensory nerves disruption), tissue dehiscence, membrane collapse and exposure; making implant surgery prolonged and more distress for the patients (González-García et al., 2011).

1.8 Piezosurgery or piezoelectric bone surgery

Piezosurgery is an ultrasonic bone-cutting system that has been use as an alternative technique from the conventional drilling system, allowing selective cutting of bone, causing minimal trauma at the time of the operation. The term "piezo" comes from the Greek word piezein, which means, "to press tight, squeeze". Jacques and Pierre Curie first discovered that applying pressure on various crystals, ceramics and bone, created electricity. (Dorland ,2007) described piezoelectric material as a ceramic or crystal material that can generate an electric potential in response to mechanical stress. The microvibrations created by piezoelectric effect in certain ceramics and crystals, deform on passing electric current through them, resulting in the material expanding and contracting, leading to an ultrasonic vibration. The vibrations obtained are amplified and transferred to a tip that when applied with slight pressure on bone tissue results in cavitation phenomena. The ultrasonic frequency usually ranges 24–36 kHz, capable of cutting mineralized tissue in dental applications. (Baker et al., 2012; da Silva Neto et al., 2014; Thomas, Akula, Ealla, & Gajjada, 2017).

Wolff's law states that bone remodels per functional demands, producing a signal which controls bone remodeling. Several researches have been conducted to help us gain insight into the nature of these stress-generated potentials. (Stacchi, Vercellotti, Torelli, Furlan, & Di Lenarda, 2013)

The cutting of hard tissue with ultrasonic vibrations using the piezoelectric effect was first described by Catuna in 1953 and then by Volkov and Shepeleva in 1974. Vercellotti introduced piezosurgery concept in 1988, offering advantages over the use of conventional bone osteotomy systems. Piezoelectric surgery improved precision, selective cutting, minimal damage to soft tissues, reduced bleeding and the absence of heating since it contains an internal cooling pump (Baker et al., 2012). The introduction of ultrasonic aids in implant surgeries have pathed to new possibilities which extend from, soft tissue debridement, smoothening of root surfaces, bone grafting, implant site preparation, removing an implant, sinus lifting procedure, retrograde root canal preparation, apicectomy, cystectomy, extraction of ankylosed teeth, and orthodontic surgeries (Thomas et al., 2017).

Di Alberti et al explain that the use of piezo promotes biological effects on odontoblast-like cells, osteoblasts and osteogenic cells, which might be of benefit in implants osseointegration. In this study, it was observed better bone density and osteogenesis after piezo was used, especially during early postoperative period (Di Alberti, Donnini, Di Alberti, & Camerino, 2010).

1.9 Minipig as animal model in implant research

Animal studies constitute the core component of histologic-histomorphometric investigation in implant dentistry. Determination of whether an implant system can be released for human use, the data collection from animal studies is paramount for the determination of the conformity of a new biomaterial or new designed implant models. The animal model selected must resemble the bone architecture of a human jaw bone, so a comparable healing can be obtained. Mandibular and Maxillary bones in pigs/minipigs are amount the commonly used for translational dental implant studies. They share anatomic and physiologic similarity characteristics with humans. The bone mineral content and density of pig have a dense trabecular network and a similar lamellar bone structure comparable with the human. The bone regeneration rate in pigs has been found to be closer to humans than dogs. The only disadvantage of this model is the need for teeth extraction before implant placement. (Erdogan, Üstün, Tatli, Damlar, & Daglıoglu, 2013; Murat Cehreli, n.d.)

Minipigs that are used in research today were developed from selective breeding, out of a need for a smaller and thus more manageable version of the domestic pig. There is a wide variation in size and weight, which influence in the amount of medication needed for testing. These animals are bred for specific laboratory research. Minipigs are larger and grow faster than dogs. The most common breeds used in the United States are Hanford, Yucatan, Yucatan micro, Sinclair and Göttinger. The last one, can reach between 7-9 kg in 4 months. They are omnivorous and most behavior is directed towards eating and rooting. Their teeth project laterally from the gums and numerous tubercles make the occlusal surface of the molars irregular, which area ideal for food crushing. An adult Minipig has a total of 22 permanent teeth can be observed, distributed in each side mandible or maxilla 3 incisors, 1 canine, 4 premolars and 3 molars (Figure 1.1). Their eyesight is good but it is their sense of smell that is the most highly developed and rooting behavior is the primary means of food searching (Swindle, Makin, Herron, Clubb, & Frazier, 2012).

Since pigs have short and sparse hair and no sweat glands they are sensitive to temperature levels, making them unable to regulate their body temperature well so if they are used for surgical research their body temperature should be monitored and regulated. They enjoy food, attention, and toys and like to pile up next to each other in the pen, which make them in a very social animal. Swine's anatomy and physiology are like humans, especially in cardiovascular, pulmonary, skeletal, and integumentary systems. These similarities have made swine the primary species of interest as organ, tissue, and cell donor species for xenograft transplantation procedures (Bollen, Hansen, Rasmussen, & Suckow, 2000).

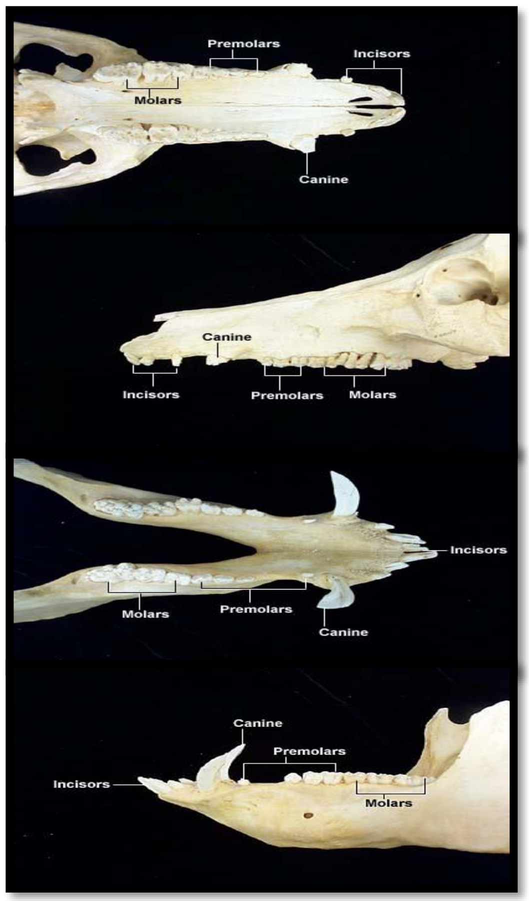


Figure 1-1: Minipig Anatomy

Figure 1: Pigs Mandibular and Maxillary anatomy. "Dental Anatomy of Pigs". M, Rouge. http://www.vivo.colostate.edu/hbooks/pathphys/digestion/pregastric/pigpage.html. Reprinted with permission from Richard.Bowen@colostate.edu.

2. HYPOTHESIS AND OBJECTIVES

Ultrasonic effect on bone healing has been proven to be beneficial after fractures and surgeries, due to promoting remodeling/modeling effects. Some studies have observed increases in bone density in comparison to conventional drilling and a rapid bone formation, which increases the chances of osseointegration. Furthermore, a press-fit wedge shape implant could be beneficial for narrower edentulous ridges in conjunction with the piezoelectric benefits. Therefore, we hypothesized that implant site preparation with piezoelectric drills plus the use of non-cylindrical implants could have an equal or better effect on stability measurements compared to cylindrical implants and conventional drilling.

The aims with this study are as follow:

To compare non-cylindrical implants using piezoelectric instruments for osteotomy (Piezoimplant) with cylindrical implants with conventional drilling (Nobel Biocare™):

i. Compare implant's initial stability with conventional drilling and piezoelectric osteotomy.
ii. Determine stability at 4 weeks, 8 weeks and 12 weeks in control and test group.

iii. Compare mandible and maxillary differences in measurements between control and test group by stability measurements.

3. MATERIALS AND METHODS

3.1 Animal Model

The study protocol was reviewed and approved by the Boston University Institutional Animal Care and Use Committee before study initiation. Three adult female Göttinger miniature pigs' (2-4 years, 40- 50Kg) were purchased from the breeder (Göttinger Minipigs, Marshall BioResorces, North Rose, NY). The Minipigs were housed separately in metal pens throughout the study, and were maintained at a temperature of 24°C ± 2°C and a relative humidity of 55%. The animals were fed a regular diet and were placed on a semiliquid diet for 2 weeks after each surgical procedure.

Maxillae and mandibles were used for the surgical implantation of the cylindrical implants and the prototype piezoimplants. Minipigs were selected to ensure adequate alveolar ridge size and height for implant placement. Each minipig received 12 implants in total and each side had one group, conventional cylindrical implants (Nobel Parallel Conical Connection by Nobel Biocare® 4.3 x 10mm as control side) and the contralateral side the Piezoimplant (prototype 12 x 5 x 2mm as test side) developed by the Biomedical Engineering Department from the

College of Engineering at Boston University in collaboration with the Fraunhofer Center for Manufacturing Innovation.

Minipigs were treated with antibiotic therapy Clindamycin 11-33 mg/Kg PO BID for 3 days as pre-medication. Pre-operative bloodwork (CBC/Chemistry profile) and overnight fasting (8 to 10 hours). Pre-anesthetic agents used: Telazol 5 mg/Kg IM plus Xylazine 2.2 mg/Kg IM for induction. Glycopyrrolate 0.01 mg/Kg IM was given to maintain heart rate and reduce secretions. After these an IV catheter was placed in the auricular vein (for IV fluid anesthesia). Animals were intubated and maintained on Isoflurane in 100% Oxygen. Buprenorphine IM and Meloxicam 0.2mg/Kg IM were given at least 30 minutes' prior surgery. Intra-operative maintenance included: IV saline or lactated ringers: 10 ml/Kg/hrs., and warm lamp for body temperature stabilization. Extra oral radiographs were taken before the surgical procedure. The veterinary technicians monitored vital signs: Heart rate, Blood pressure, Pulse, Respiratory rate, SpO2 and body temperature every 15 minutes.

Each animal was intra-orally rinsed with chlorhexidine. Maxillae and mandibles 4 premolars and molars (except for M3) were surgically extracted *(Figure 3.1 and 3.2)* using infiltrative Lidocaine 2% with epinephrine 1:100.000 local anesthetic. Intrasulcular incisions were made buccal and lingual on each quadrant; full mucoperiosteal flaps were reflected; teeth were extracted with two or three dental sections using a high-speed drill; these were luxated and extracted using elevators and forceps. Vycril® 4.0 synthetic resorbable suture was used on each surgical site. Post-procedure monitoring (weight, swelling, discharge and diet) was performed for 72 hours by technicians in the animal facility. Medications post-operative included: Meloxicam 0.2 mg/Kg PO q24 for 3 days, Clindamycin 11-33 mg/Kg PO bid (7-10 days), Fentanyl transdermal (1-5 mcg/Kg) for 72 hours.

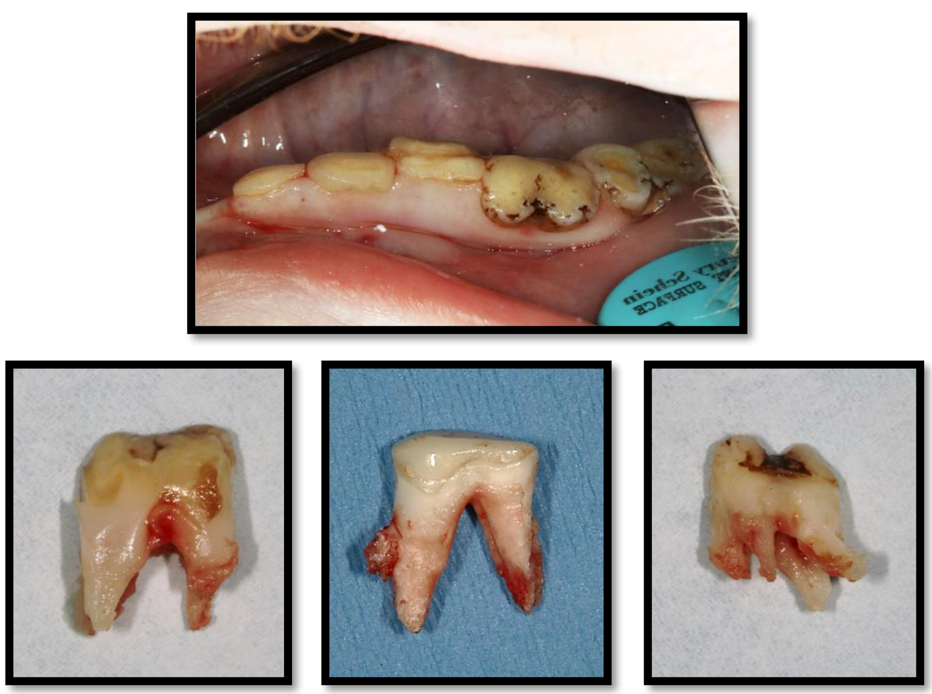


Figure 3-1: Intraoral photographs of the mandibular teeth and individual premolars and molar after extraction.

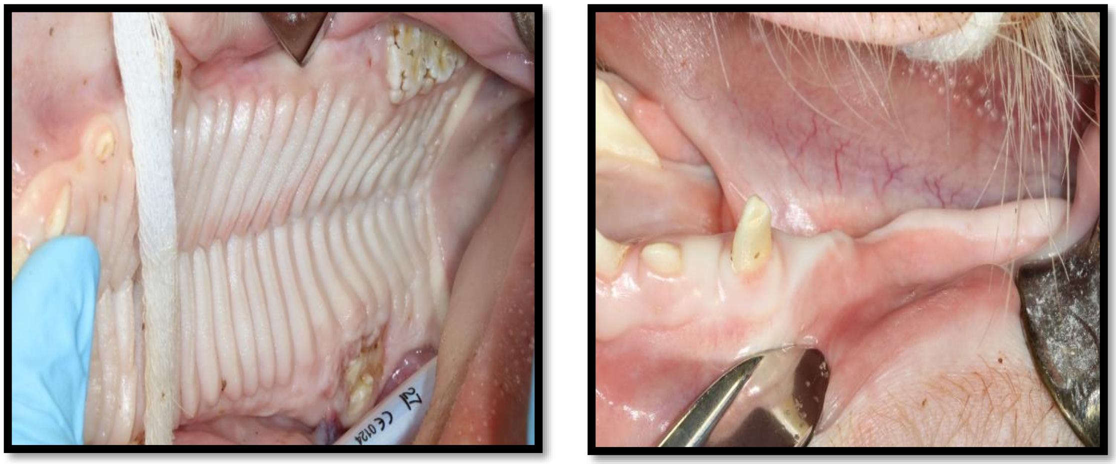


Figure 3-2: Intraoral photographs of mandible and maxilla. 3 months healing after extractions.

Three months after extractions (Figure 3.3), the placement of the conventional cylindrical implants and Piezoimplants was performed. Same settings for general anesthesia and medications were maintained for the animals as on the first surgery. Surgical treatment of the maxillary and mandibular edentulous sites started by a midcrest incision and a release incision, anteriorly sloping into the buccal vestibules. The periosteum was reflected exposing the underlying alveolar bone.

For the cylindrical implants, as control site, Nobel Biocare® implant surgical kit was used to preparer the three osteotomies. 4.3 by 10 mm internal connection implants were placed mesially to the last molar (M3), around 21 mm were measured for the placement of the osteotomies. In total 6 were done on each minipig to house the conventional implants. Each of them were screwed in osteotomy using up to 45 Ncm of torque and ratchet manually, cover screws RC (yellow) were placed on each implant before suturing with Vycril® 4.0. *(Figure 3.3)*

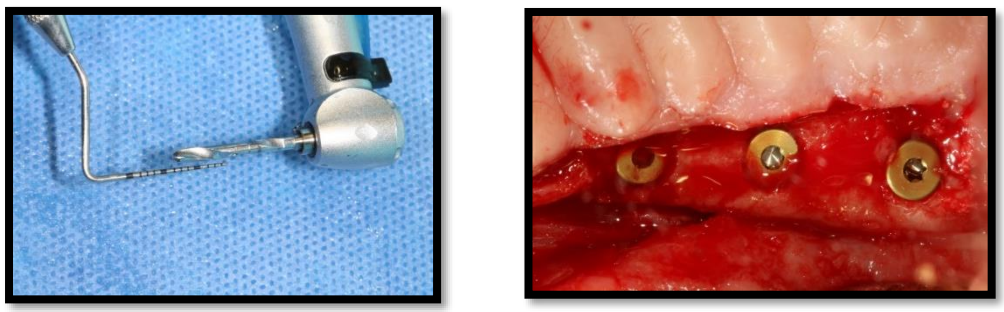


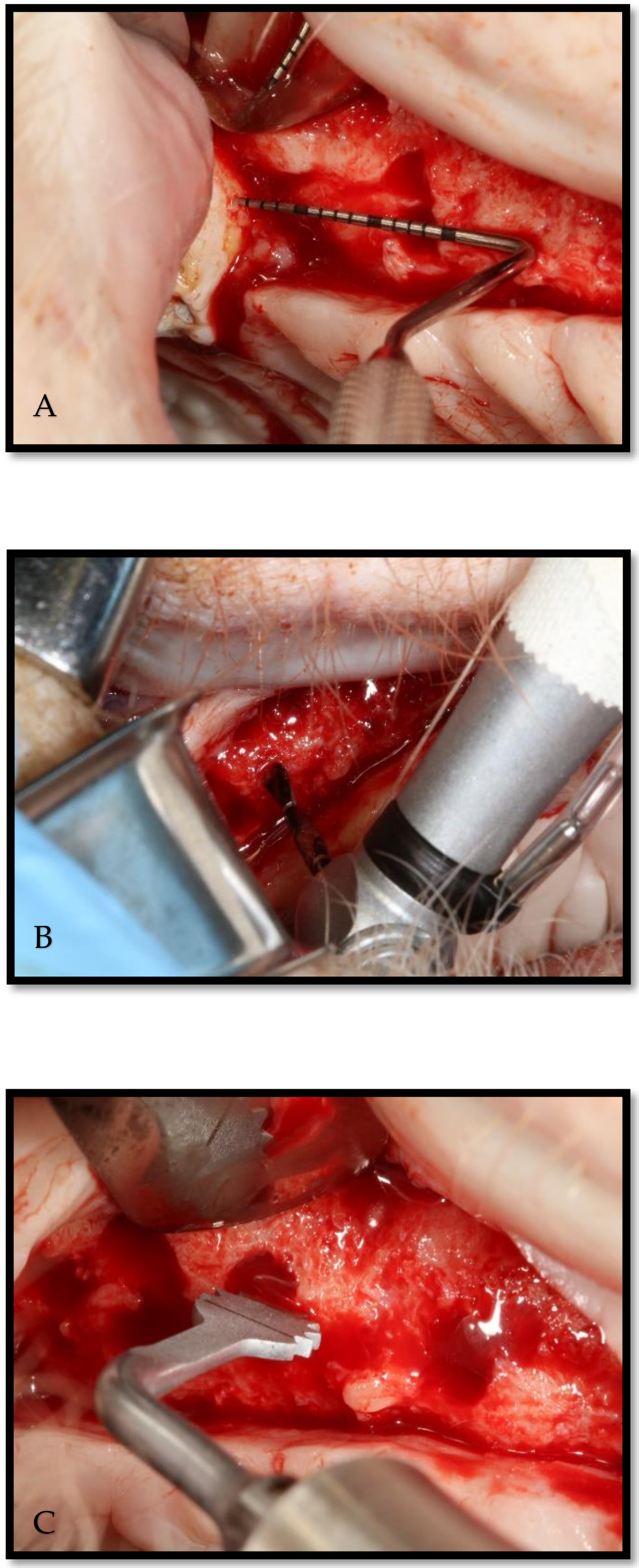
Figure 3-3: Conventional cylindrical drill and cylindrical implant placement

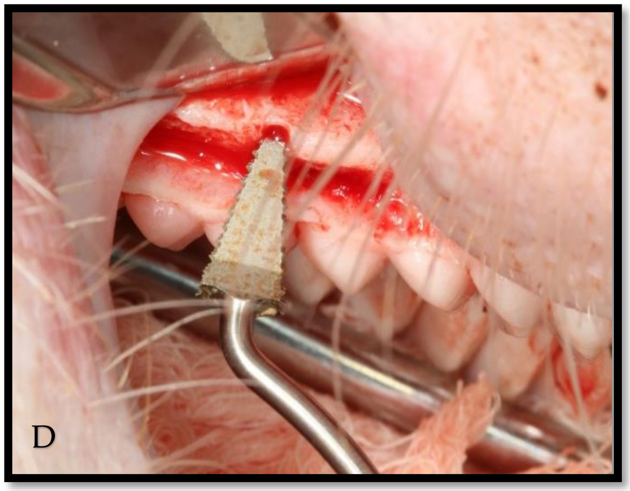
For the Piezoimplants, the piezoelectric knife (frequency range of 28 to 36kHz Piezotome 2, Satelec, Acteon group, Merignac France) was used after flap was reflected on the opposite maxillary and mandibular edentulous ridges. Each implant was sandblasted with a MCD Apatite Abrasive, and sterilized in Autoclave®. Initial osteotomy was done using a 2mm diameter cylindrical pilot drill in the midcrest bucco-lingually position, until reach 10mm in length, after this a flat piezo insert of 5mm width was used as initial mesio-distal drill, and a lesion of 3 mm depth was made. Subsequently, another piezo insert of 2mm width was used to drill deeper. *(Figure 3.4 B and C)*

Then the Piezoimplant shape tips were used, the first insert which cut in a mesio-distally direction into the bone and the second tip enlarged in a bucco-lingually direction *(Figure 3.4 D and E).* Having reached the desired shape, the Piezoimplants were finally tapped in with a surgical mallet and an Offset handle (Bicon® Instruments: Surgical Mallet, part number 260-081-165. Offset handle, part number 260-101-009) until the implant bodies were fully embedded in bone. In total 6 Piezoimplants were placed in each animal.

In control and test side the implantation was stopped after the implant shoulder was completely inserted into the bone and the required values for stability were registered. Afterwards, a Periotest instrument (Siemens Co, Bensheim, Germany) was used to evaluate and measure Piezoimplant primary stability prior to suturing *(Figure 3.4 G, H and I)*

Animals were scheduled to be sacrificed after 4, 8 and 12 weeks of healing time. Two surgeries were planned to be performed a month apart to ensure that there would be two animals together until the end time point. The animals were euthanized with the same drugs used in preparation for surgery. Once sedated, a catheter was placed in the ear vein and an overdose of pentobarbital,138mg/kg of euthanasia solution was administered IV (Fatal Plus®). Maxillae and Mandibles were removed en bloc using bone saws under saline solution. Specimens were fixed in secure labeled plastic jars with 10% formalin and solution was changed three times every 3 days.


A
B
C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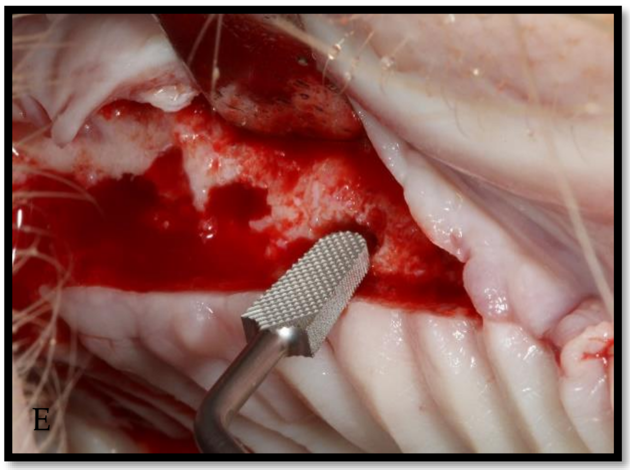
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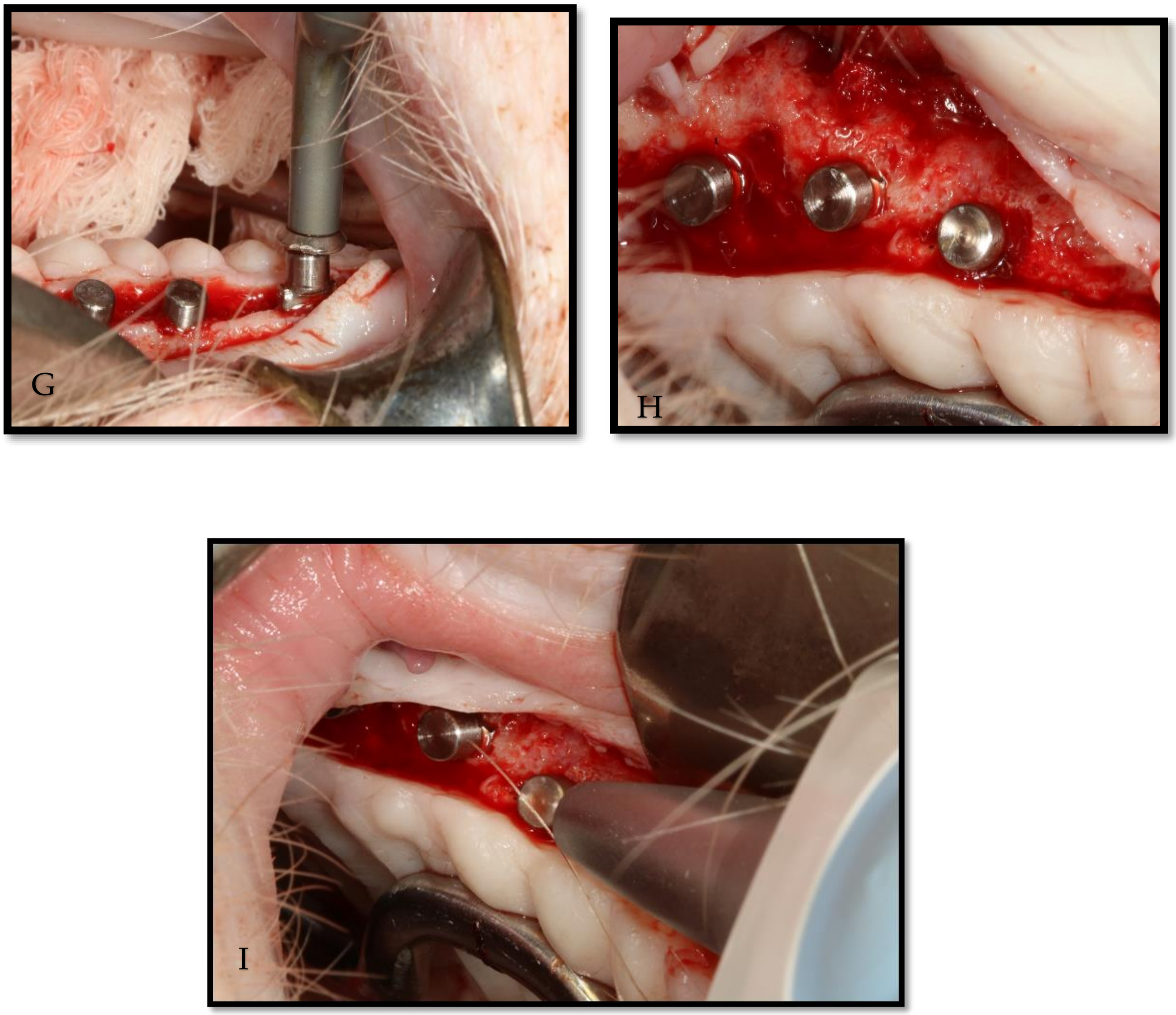


Figure 3-4: Piezosurgery for non-cylindrical implants (piezoimplants).

(A): Measurement from the last Molar to locate the position of the three implants/ (B): Pilot cylindrical Drill of 2.0 of diameter/ (C):Piezoelectric tip of 5mm for a mesio-distal cut/ (D):First piezo insert for the non-cylindrical osteotomy for mesio-distal enlargement/ (E):Second piezo insert for bucco-lingual osteotomy preparation/ (F):Insertion of the non-cylindrical implant (Piezoimplant)/ (G) surgical mallet and handle to press-fit the implant/ (I): Periotest for stability measurements.

3.2 Clinical Macroscopic and Radiographic assessment.

Surgical outcomes were assessed after extractions and later after surgical implant placement, using an external radiographs machine at the Animal Facility (Diagnostic Imaging system, Inc CR4000. Ultra1002040), *(Figure 6)* it was used also after each euthanization to assess each block of bone-implant samples. Stability evaluation was done postmortem using a Periotest M device (Medizintechnik Gulden, Manufacture of Periotest) and the measurements were registered and archived for each experimental and control side. At control side, a Nobel Healing abutment of 3mm high was used for the evaluation with the Periotest device, and for the test side the head of the Piezoimplants was used as testing site for stabilization.

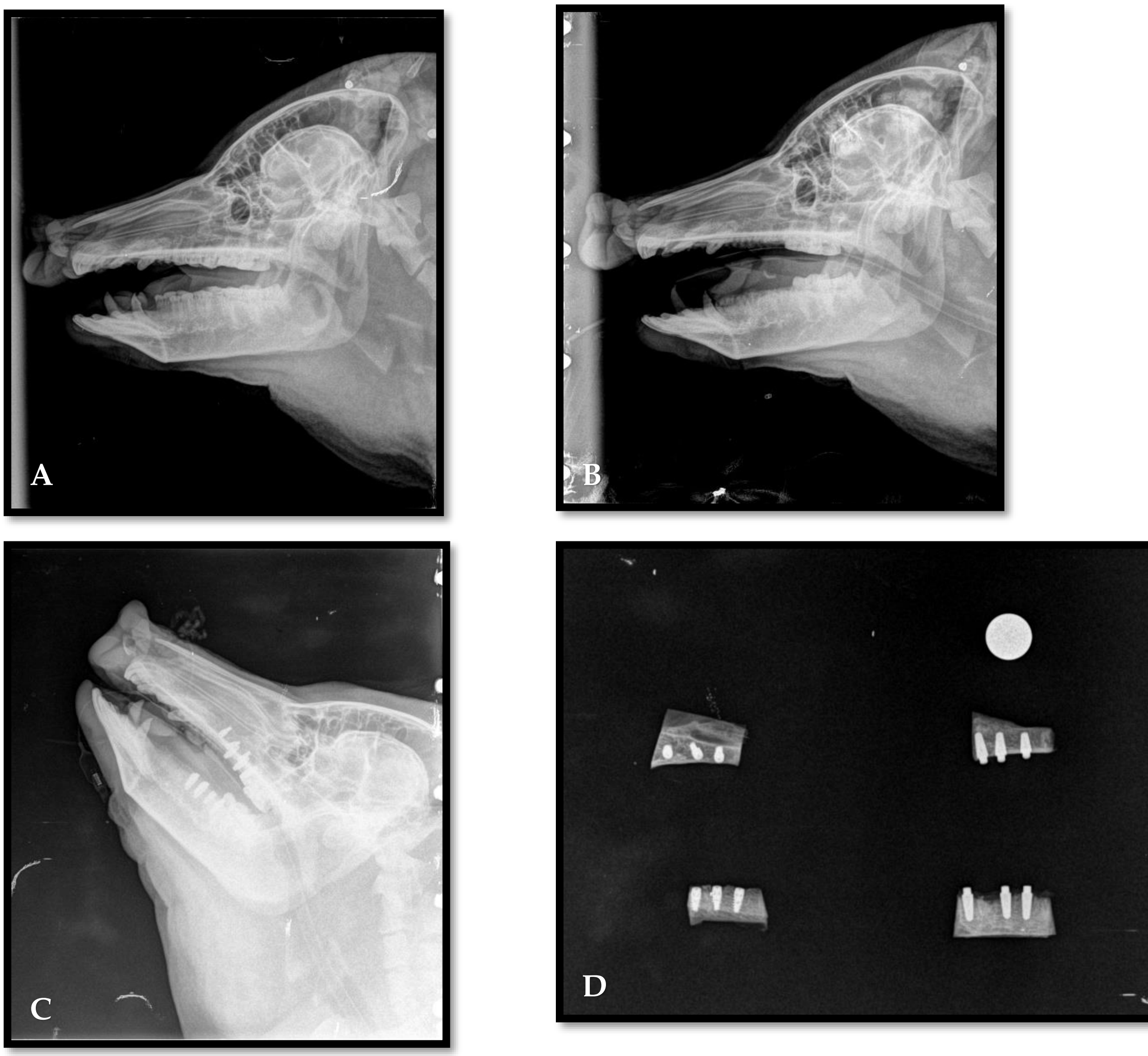


Figure 3-5: Radiographical assessment of the Minipig.

Radiographical assessment (A): before teeth extractions/ (B): after teeth extractions/ (C): after implant placement/ (D): samples post-euthanization.

4. RESULTS

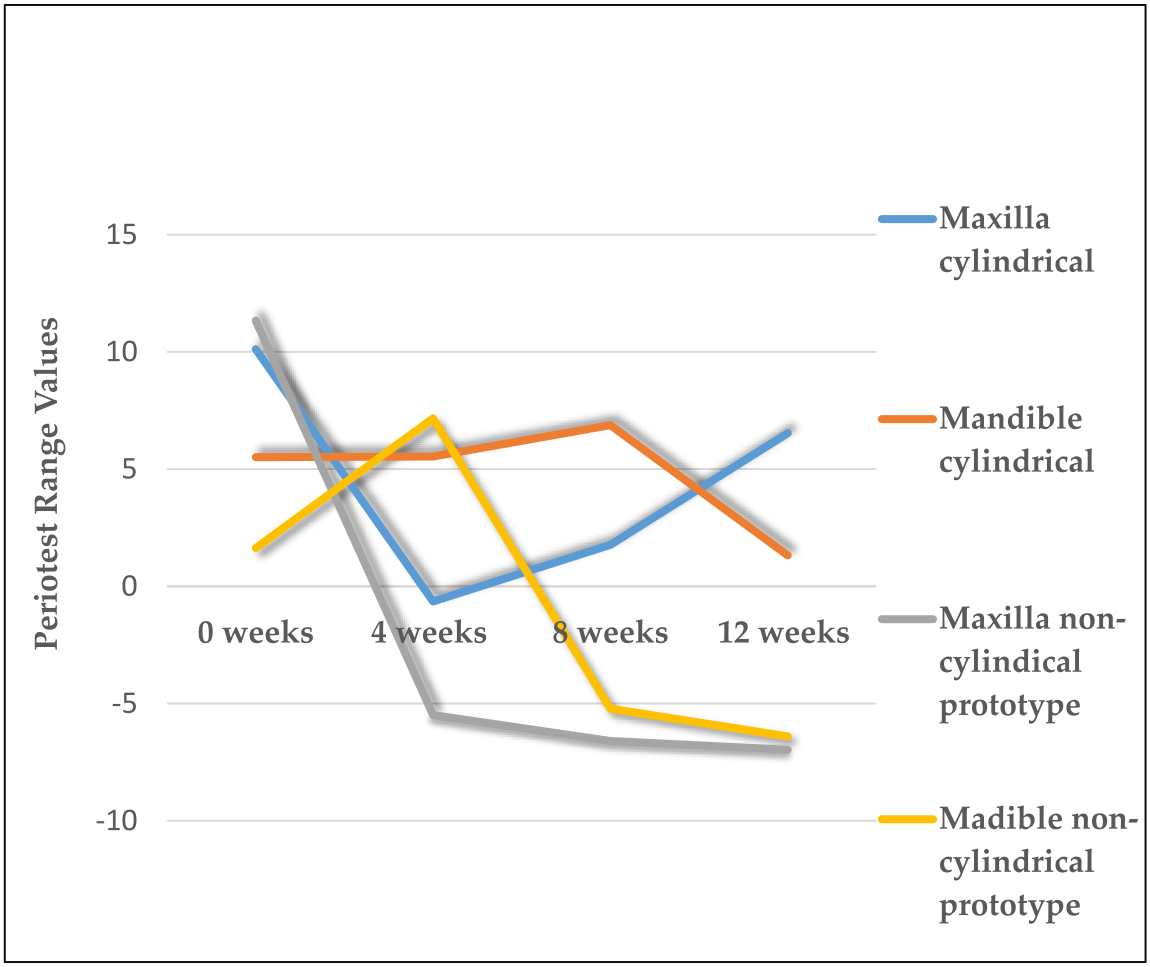
4.1 Clinical evaluation.

After sample collection, soft tissue on surface of bone blocks was removed. All implants showed high survival rates. Only minimum crestal bone loss was observed in localized areas and one piezoimplant perforation into the sinus floor.

4.2 Assessment of stability.

To determine the stability of the implants, a percussion test using a Periotest device was performed at baseline (day of placement) and repeated at the day of euthanization for each minipig (4, 8 and 12 weeks) in the control group (cylindrical implant) and in the test group (non-cylindrical) on mandible and maxilla respectively. Significantly higher Periotest values were measured in the test group in mandible at week 4,8 and 12, and only in maxilla at week 8 and 12. Comparing the stability between cylindrical implants and non-cylindrical, higher values could be assessed in the non-cylindrical areas.

Table 2: Stability Test of non-cylindrical vs cylindrical implant

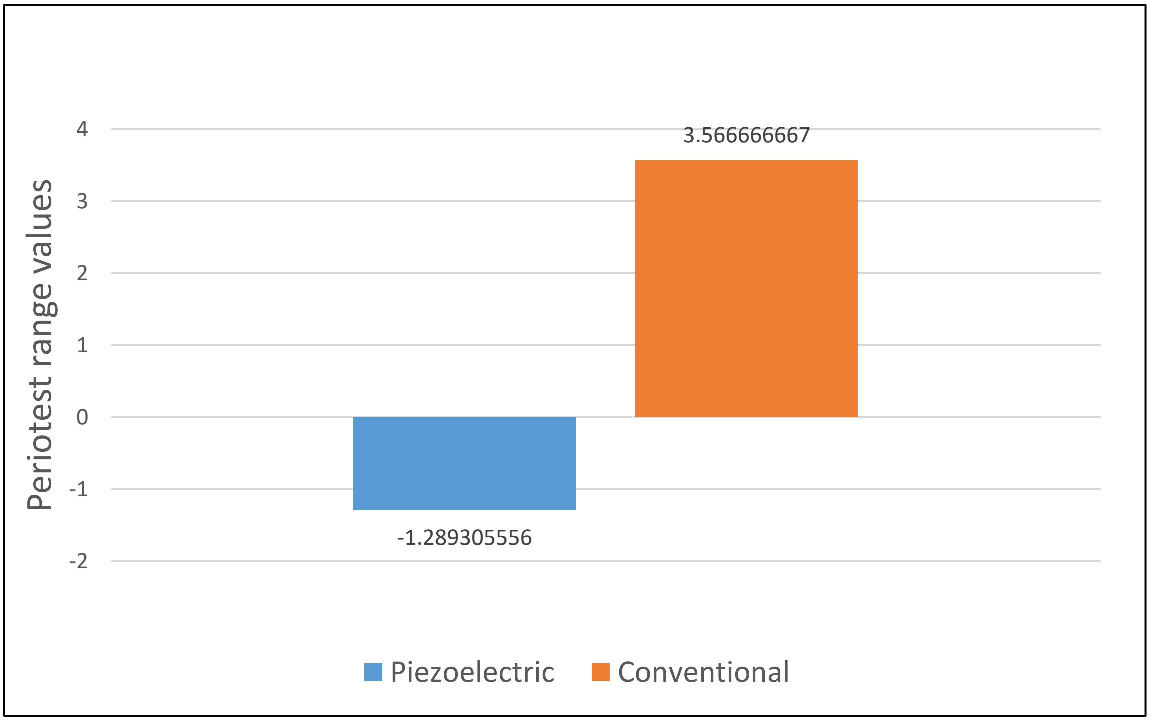


4.3 Assessment of Average Periotest Values.

For the cylindrical implants placed following conventional rotary ostectomy (n =18), the average percussion test value was 3.56 PRVs. Whereas for the non-cylindrical implants placed following piezoelectric ostectomy site preparation (n

= 18), the average PRV values was -1.28. Statistical differences were reported when compared the two groups.

Table 3: Piezoelectric vs conventional implant site preparation. Average Periotest Values



4.4 Analysis of variant (ANOVA) test results

A generalized linear model for repeated measures was used to determine the effect of each osteotomy and implant design at every time point. The difference of design plus osteotomy (test vs control group), time point (week) and location was studied by ANOVA to account multiple implants within the individuals. The R-square is only 0.51, which indicates only 51% of the response variability can be explained by the fitted model. Among all the 3 factors, group (test vs control) is the most significant one, followed by time point (week). Location significance is the least among the three factors.

Table 4 Prediction plot for stability test

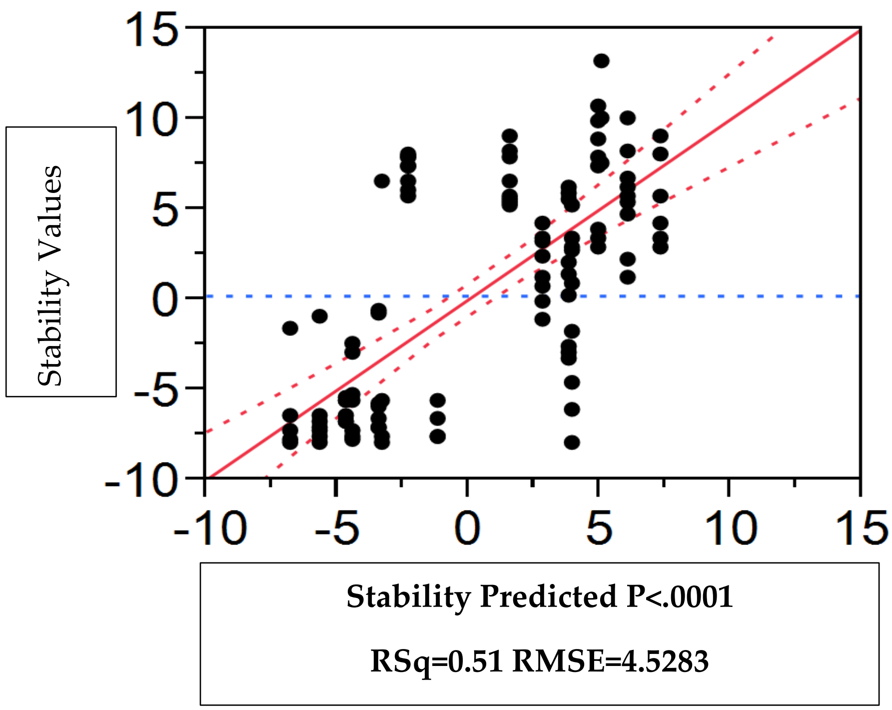


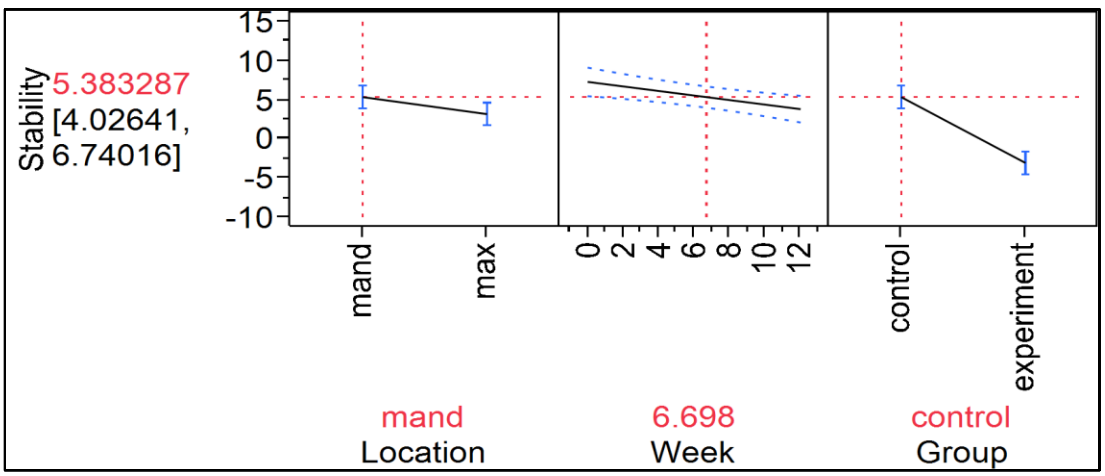
Table 5 Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Group[control]	4.2092575	0.399088	10.55	<.0001*
Week	-0.29046	0.094965	-3.06	0.0027*
Location[mand]	1.088201	0.398748	2.73	0.0073*

Table 6 Summary of Fit

RSquare	0.510512
RSquare Adj	0.498764
Root Mean Square Error	4.52826
Mean of Response	0.192248
Observations (or Sum Wgts)	129

Table 7 Linear regression of the three factors: Group, Time point (week) and Location.



5. DISCUSSION

Dental implants in edentulous areas often required reconstructive bone graft surgeries to compensate for the bone resorption that occurs after teeth are extracted. Patients often reject these procedures due to cost, morbidity and time involved.

Piezoelectric surgery produces a selective cutting action that does not harm soft tissue structures and results in favorable osseous repair, increased bone density and remodeling.

The idea to introduce a new type of endosseous implant in conjunction with the osteotomy technique using piezoelectric properties as described previously, would reduce problems encountered in knife-edge ridges.

Additionally, the long-term success of an implant requires rigid fixation of the implant within the host bone site. The threads allow mechanical interlocking within the bone. There are studies that suggest that the quality of implant osseointegration and stability is dependent to a large extend on the geometric implant design. Also, certain implants designs may promote osseointegration, providing local mechanical environment for a faster healing and a stable fixation between bone and implant. (Meyer et al., 2004)

The aim of this study was to evaluate changes in the stability of cylindrical implants inserted in sites prepared with rotatory instruments versus non-cylindrical implants inserted in sites prepared with piezosurgery technique.

Piezoelectric technique was introduced to decrease the difficulty in cutting thin or delicate bone structures with precision cuts. Insertion of implants in areas of thick bone with small alveolar ridge shaped was facilitated by the use of piezoelectric tips, which have an effective cut in comparison to the rotatory conventional drilling (da Silva Neto et al., 2014). Other studies as well, have compared the effect of an ultrasonic insert and a rotatory bur (Tomaso Vercellotti et al., 2005) observing the bone healing proceeded best when bone was removed by piezoelectric knife.

Cutting efficiency with rotatory drilling requires normally a good grip and manual pressure, which may lead to fenestration as results of the rotation and an atrophic alveolar ridge.(da Silva Neto et al., 2014)

Currently there are several methods to assess implant stability and osseointegration. The method that has confirmed more sensitivity is the resonance frequency analysis or ISO (Atsumi, Park, & Wang, 2007), which requires a special component to be attached to the cylindrical implant. For a non-cylindrical implant, the attached component need it for analysis of stability is not available, reason why, it was selected another stability test using a Periotest instrument that applies forces with a hammer using an electromagnetic driven and electronically controlled tapping metallic rod that needs to contact the implant neck or abutment with a damping motion. This tapping signal is converted to a Periotest range value; this technique is rapid, straightforward and easy to achieve.

Immediately after implant placement, Periotest range values were not statistically significant different in cylindrical and non-cylindrical sites on mandibular and maxillary sites. However, at 8 weeks after implant placement, a clinical difference in Periotest range value was found between the cylindrical and non-cylindrical implant groups.

The reduction or negative values were more pronounce in the group of non-cylindrical/piezoelectric osteotomy showing a better and increased stabilization. Significant osseous response to piezoelectric technique and non-cylindrical implant was observed, also been reported in previous studies (T. Vercellotti, 2004);(Tomaso Vercellotti et al., 2005);(da Silva Neto et al., 2014). The increased of stability was observed in both groups, although the non-cylindrical/piezoelectric group was still above of the cylindrical/conventional drill group.

In the research conducted by Da Silva Neto et al. they observed that the success of the piezoelectric technique may be related to the accommodation of the bone after compression during installation of the implant and more favorable biological change in early bone remodeling.

Using the analysis of variance (ANOVA) it was observed that only between control and test group had significant statistics ($P<.0001$) compared with week of testing and location. The possibilities to use a wedge-shape implant design in combination with piezoelectric technology for the site preparation when the residual ridge has severe deficiencies demonstrate in this study that, the stability test achieved clinical and statistical differences between test and control group. The use of piezoelectric technology has been used to minimized the trauma and the risk of ridge fractures on site preparations (T. Vercellotti, 2000).

As a conclusion, non-cylindrical (piezoimplants) showed significant improvement on stability in maxilla and mandible compared to the cylindrical implants.

According to the PRVs tests, the non-cylindrical implant showed better primary stability on maxilla and mandible since week 0 (baseline) until week 12.

Further analysis is needed using μ-CT and histological evaluation to evaluate bone-implant interface and formation in comparison between the two implant models.

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7. CURRICULUM VITAE

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