Alveolar Bone Characteristics of Well-Controlled Versus Poorly-Controlled Diabetic Patients

A Thesis submitted to the faculty of the university of Minnesota by

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This thesis is dedicated to my parents Haim and Neta, my brother Alon and sister Nurit.

Their love, support and encouragement made this possible

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1. Background and Literature Review

1.1 Diabetes Mellitus as a metabolic disorder

Type 2 Diabetes Mellitus (DM2) is a metabolic disorder that is associated with defects in insulin utilization. DM2 is very prevalent in the adult population affecting approximately 29.1 million people in the US alone, which is roughly 9.3% of the population [1]. DM2 affects patients by increasing the risk to macrovascular and microvascular complications such as stroke, coronary artery disease, neuropathy and seem to affect long bones as well [2]. It is considered a risk factor for dental implant complications [3]. These alterations may impair response to surgical trauma in patients with inadequate diabetic control [4,5]. In order to assess ones diabetic levels, glycosylated hemoglobin levels (HbA1c) are utilized as surrogates for the assessment of diabetic control during pre-surgical treatment planning in the medical field [5,6]. According to the American Diabetes Association, well controlled individuals have an HbA1c $\leq$ 5.7% while pre-diabetes ranges from 5.7-6.4% and individuals diagnosed with diabetes includes HbA1c values of 6.5% and higher. DM2 had been associated with high bone mineral density and also higher bone fracture rates when compared with non diabetic individuals [7].

According to multiple studies, high glucose levels may lead to accumulation of Advanced Glycosylation End Products in the organic bone which may lead to collagen production distortion that could result in a more fragile bone formation [8]. The most frequently encountered complications in implant dentistry in patients with poor diabetic control are soft tissue associated [4,9].

1.2 Previously published evidence on Type II DM bone histomorphometry

Klein *et al*. [10] in the early 1960s, was the first to report a histomorphometrical analysis on bone in patients with Type II DM. They noted an increase cortical surface area in the rib bone. Conversely, multiple authors subsequently found a decrease in bone formation depicted by a decrease in mineral apposition and rate of bone formation suggesting that Type II DM promote osteoblastic activity dysfunction [11,12]. Manavalan *et al*. [13] investigated iliac crest bone biopsies from six Type II DM postmenopausal women and found reduced osteoid, osteoblast and mineralizing surfaces when compared to iliac crest biopsies from six non-diabetic postmenopausal women.

1.3 The role of Advanced Glycation End Products

While bone mineral density is the determinant of bone strength, the collagen fibers are the major contributors for tensile strength, ductility and toughness [14]. Among the influencing factors affecting these properties are the tissue turnover rate and the cellular activity. Collagen cross-link formation can be classified into either lysyl oxidase regulated or oxidation induced Advanced Glycation End Products (AGEs) cross-linking. Saito and Marumo reported that increased AGEs levels lead to poor bone quality formation with increased risk of fracture [15]. AGEs relate to increased osteoclastic activity in postmenopausal patients with osteoporosis [16, 17]. Valcourt *et al,* have found that AGEs decrease bone resorption by altering the structural integrity of bone matrix protein while also inhibiting osteoclastic differentiation [18]. AGEs tissue levels seems to be regulated by glycemic control, oxidative stress and the tissue's life span [19,20,21]. The onset of Type II DM is accompanied by a marked increase of AGEs as well as a decrease in bone quality associated with minimal change to collagen content and bone mineral density [2]. Consequently, it can be concluded from the preceding literature that AGEs are associated with low-bone formation and turnover [23].

1.4 Present histological data

La Fontaine *et al.* [24] histologically evaluated bone biopsies from feet and ankles of non-diabetic and Type II DM patients. They found that the diabetic group formed mostly lamellar bone, with less osteocytes when compared to the non-diabetic group. A few lacunae were empty and the marrow spaces were filled with adipose tissue. The trabeculae in the diabetic group were thinner and fewer. Minimal bone remodeling was present and fewer osteoblasts were present. They concluded that the diabetic group displayed less overall cellularity which might impair the reparative process.

1.5 Changes to bone microstructure

Type II DM has been associated with increased mineralization of cancellous bone as well as with increased cortical porosity [23,25,26]. The severity of this cortical porosity seems to be associated with the extent and duration of the diabetic disease.

However, newly diagnosed individuals presented with a similar risk of bone fracture risk as healthy individuals [27,28]. It has been well established that circulating osteogenic precursor cells are down regulated in Type II DM while mesenchymal stem cells are more likely to differentiate to adipocytes, yielding

an increase bone marrow adiposity in Type II DM patients when compared to healthy control subjects. [13,29,30].

1.6 Diabetes Mellitus and Possible Post Surgical Complications

A large retrospective study of total joint replacement retrospectively evaluated the results of 6,088 hip replacement surgeries in diabetic patients that presented with increased HbA1c levels [31]. It was found that when comparing different HbA1c level thresholds, a decrease from 7.5% to 6.5% resulted in a small 2.7% reduction in post-surgical complications but was associated with 18.8% unnecessary delays in an attempt to achieve better glycemic control. Tawil *et al.* investigated the effects of Type II DM on dental implant survival and the associated complications in 90 patients. It was concluded that an 8% rate of soft tissue complications was observed in patients with HbA1c values between 7% and 9% when compared to a rate of 6% in patients with HbA1c levels less than 7% [32]. Another study by Oates *et al*. reported that individuals with HbA1c levels $\geq$ 8.1% exhibited compromised early implant stability [3]. However, other published studies have not consistently shown significantly compromised survival rates in poorly controlled diabetics [4,32,33,34].

1.7 Diabetes Mellitus and Dental Implants

Therapeutic management of DM2 has been based upon the regulation of elevated levels of hyperglycemia. Significant evidence exists to support the placement of implants in diabetics with HbA1c levels within the normoglycemic range [3,4,33,35]. However, recent publications of clinical trials have addressed the success of implants placed inn patients with poorly controlled diabetes. The harmful effects of hyperglycemia on implant integration and survival has been attributed to microvascular complications in the alveolar bone that lead to compromised blood supply and decreased bone density [36]. Nonetheless, patients with DM2 that cannot achieve optimal glycemic control (HbA1c<7.0%) may represent over 50% of the diabetic patient population [37]. Thus, the histological identification of variations in bone vascularity and morphology may improve the efficacy of implant treatment modalities in this patient group thereby increasing the Oral Health Related Quality of Life for this large portion of the population.

1.8 SLA active implant characteristics

Variations in implant surface texture and composition have been developed to enable poorly controlled diabetics to gain access to implant care while minimizing post operative complications, researchers have identified improved implant characteristics that can enhance implant osseointegration in pre-clinical studies performed with diabetic animal models. Chemically modified, micro-rough, hydrophilic (SLActive®) titanium implant surfaces have been shown to promote bone formation and accelerate osseointegration of dental implants placed in diabetic animals [9,38]. It was noted that these implants promote angiogenesis and that the increased angiogenesis is directly correlated with new bone formation [39]. It has been hypothesized that this enhanced biologic response is due to the biocompatibility and hydrophilicty of the surface that actively attracts blood and becomes populated by progenitor cells and growth factors that improve stromal cell differentiation [40]. A recent advancement of this chemically modified surface is the introduction of a binary Titanium-Zirconium (TiZr) alloy that is compatible with the SLActive surface treatment [41]. In vitro pre-clinical studies have shown that hydrophilic TiZr implants (Roxolid®) have a dual benefit over titanium implants with SLActive modified surfaces. TiZr implants have a higher tensile strength that enables use of narrow diameter implants without risk of implant body fracture. These implants also

enhance bone in-growth compared to chemically modified titanium surfaces [41,42].

2. Rationale, Hypothesis and Specific Aims

2.1 Rationale

Pre-clinical studies support the use of hydrophilic TiZr implants (Roxolid®). It has been speculated that the more hydrophilicty an implant surface is may lead to greater vascularity during the implant integration period with enhancement in growth of bone on the implant surface as previously shown in animal studied [9,38]. Consequently, the use of the hydrophilic TiZr implant surface could enhance implant placement in poorly controlled diabetic patients and help alleviate disparities in oral health-related quality of care issues among diabetic patients.

2.2 Hypothesis

Hyperglycemia results in compromise vascularity and increased bone marrow adiposity in the mandibular bone. Therefore, hydrophilic TiZr implant surfaces (Roxolid®) that actively attract fluids and possess excellent osteoconductive properties leads to an early implant survival and success in poorly controlled DM2 patients to levels comparable to well-controlled DM2 patients.

2.3 Primary objective

Assess bone vascularity and bone marrow adiposity in well controlled diabetic patients versus poorly controlled diabetic patients by means of histomorphometric and immunohistochemical assessments.

2.4 Secondary objective

The secondary objective of this study is to compare in well-controlled DM2 patients versus poorly-controlled DM2 the initial implant stability of hydrophilic TiZr implant surfaces (Roxolid®), post-surgical pain levels, early implant survival and success rates at three years.

3. Material and Methods

3.1 Study population

The study population consisted of partially edentulous adults enrolled as well controlled type 2 diabetic patients (5.8<HbA1c≤7.0%) and poorly controlled type 2 diabetics (7.5<HbA1c<10%). Participants were recruited from patients with a diagnosis of Type II Diabetes Mellitus and presented to the Advanced Education Program in the Division of Periodontology at the University of Minnesota for implant treatment in the mandible. Participants who met the following inclusion and exclusion criteria were accepted into the study.

Inclusion criteria:

- Adult patients aged 18-85 years with a physician diagnosed DM2.
- History of DM2 for at least two years prior to enrollment.
- At least one edentulous site in the mandibular canine or posterior mandible regions.
- HbA1c >7.5% & <10% for enrollment in the test group.
- HbA1c >5.8% & ≤7.0% for enrollment in the control group.

Exclusion criteria:

- Mandibular sites that will not allow bone core retrieval due to limited alveolar bone width (ridge width <6mm, height <12mm) as confirmed by pre-operative Cone Beam Computed Topography (CBCT).
- Smokers: current, or ex-smokers with <2 years since smoking cessation.
- Patients that present with a grafted study site.
- Active periodontal disease that is not in remission.
- Medications that affect bone healing (e.g. bisphosphonates or chronic steroids).
- Patients who are carriers of transmissible disease(s) that may unnecessarily expose laboratory personnel to risks such as HIV, Hepatitis C and others.
- Participants with a physician diagnosis of osteoporosis (Z-score ≤ -2).
- Females during pregnancy or lactation and females that plan to become pregnant within the following year.
- Patients that will not agree to participate in this study or sign the consent form.

3.2 IRB approval

Initial University of Minnesota Institutional Review Board approval was received on May 21st, 2015.

3.3 Straumann LTD support

Application to Straumann LTD for research support was submitted in December 2014 and approved by Straumann LTD on February 27th, 2015. A total of 46 Roxolid SLActive® implants (Forty-two 4.1 mm x10 mm & Four 4.1 mm x8 mm) with 48 healing abutments (varying sizes) were provided by Straumann LTD for this investigation.

3.4 Patient appointments

3.4.1 Pre operative consult appointment

School of Dentistry patients who met the inclusion criteria were contacted via phone or in person to ask if they would be willing to volunteer to become a participant in this study. Information about their medical and dental history was obtained from a complete dental and periodontal examination. Any necessary dental treatment was completed prior to official enrollment into the study. All patients were asked to undergo a baseline CBCT radiographic examination of the mandible to ensure the presence of adequate bone width and height. The CBCT was analyzed by a board certified oral radiologist, Dr. Mansur Ahmad, DDS, PhD and by the operating surgeon [43]. A blood draw was performed and submitted to a CLIA-certified lab (Fairview Diagnostic Laboratories, 420 Delaware ST SE, Minneapolis MN, 55455) to confirm that the HbA1c values were within the above mentioned inclusion range. Blood was drawn from the antecubital fossa and transferred into a 3mL EDTA coated tube (Fisher Scientific, Pittsburg, PA, USA).

3.4.2.1 Implant surgical visit

Eligible patients, who met the inclusion and exclusion criteria were invited to participate in this clinical trial. A consent form and a Health Insurance Portability and Accountability (HIPAA) form were given and verbally read to each patient. Upon signing the consent form, blood pressure was taken, patients were pre-medicated with 1 gram amoxicillin. If, a patient was allergic to the penicillin group of antibiotics then a 300 mg dose of clindamycin was given instead. A 0.12% chlorhexidine gluconate intra-oral rinse was given for 1 minute prior to the start of the surgery. Patients were prepared according to the sterile protocol provided by Scharf *et al.* that included sterile gloves, implant, instruments, irrigation, gowns, drapes and masks, antibiotic coverage, head covers and peri-oral skin preparation [44]. Routinely, 2% Lidocaine with 1:100,000 epinephrine (Dentsply Pharmaceuticals, Cambridge, Ontario, Canada) was used for inferior alveolar nerve block in the operated quadrant. After giving anesthesia, a mid-crestal incision was performed with either a 12b or 15c scalpel followed by a full thickness mucoperiosteal flap reflection. If that flap reflection did not provide sufficient visibility a vertical releasing incision was placed on the buccal surface. The osteotomy site was identified with the help of the restorative dentist (Dr. E. Johnson, DDS) and was marked with a ½ round carbide bur in a

slow speed handpiece utilizing copious sterile saline irrigation. A two-piece 2.75 mm internal diameter and 3.5 mm external diameter trephine surgical bur was employed at 300 rpms under continuous sterile saline irrigation was used to retrieve a bone core (2.5x7.0 mm) from the future implant site (Figure 1).

The retrieved bone core would of otherwise been removed during implant site preparation and discarded as surgical waste. The bone core was immediately transferred to a 10% neutral buffered formalin for 12 hours and later to 70% ethanol. Bone core samples were then sent for analysis to the University of Minnesota Masonic Cancer Center, St. Paul, Minnesota (Dr. O'Sullivan). Further preparation of the surgical area continued per Straumann LTD protocol, which included preparation with a final twist drill (Twist Drill PRO, 3.5Ø) at 500 rpms under copious sterile saline irrigation. If needed, a Straumann Bone Level Tap Drill was utilized (For D-1 bone) at 15 rpms. All participants of this study received a single 4.1x10 mm Titanium-Zirconia, hydrophilic (Roxolid®) implant. The maximum insertion torque was recorded using the implant handpiece and confirmed by the hand torque wrench. The implant stability quotient (ISQ) was measured in two planes (Mesial and Buccal) three times and the average was recorded as an index of primary implant stability [45]. Any exposed threads were measured, recorded and bone grafted if necessary. The healing abutment was connected to the implant unless otherwise contraindicated by a low insertion torque (<15 N.cm). Routine interrupted Coated Vicryl 4-0 sutures were placed (Ethicon, Somerville NJ, USA) to allow for passive flap closure.

3.4.2.2 Post-operative care

Patients undergoing implant placement in the Advanced Education in Periodontology Clinic at the University of Minnesota School of Dentistry received routine post operative-instructions verbally and in written form. Ice was applied to the surgical side for the first several hours after surgery to minimize swelling. Post-operative analgesic consisted of 600mg of Ibuprofen 4 times a day for the first 3 days and then as needed for pain management. Chlorhexidine gluconate (0.12%) rinses were performed by the patient twice a day for 14 days. Following the initial pre-operative loading dose of 1,000mg of Amoxicillin a dosage of 500 mg was prescribed for three times a day for 7 days. Patients who were allergic to the penicillin family of antibiotic were prescribed with clindamycin 150 mg three times a day for 7 days. Patients were instructed to avoid brushing the surgical site and to chew on the opposite side of their mouth during the healing period.

3.4.3 One and Two week post-operative visits

Patients were seen at 1 and 2 week post-operative visits whereby adverse events were recorded. Two separate registries of surgery-related adverse events (AE) regarding known surgical risks (e.g. wound infection [defined as surgical sites presenting with active exudate] or sites with an implant failure [with evidence of an implant rejection]), and unanticipated serious adverse events (SAE) [defined as intraoral and or extraoral swelling, osteomyelitis or cellulitis] were noted and managed. Self reported pain on a 0-10 visual analog scale (VAS) was recorded with 0 being no pain and 10 being the most intensive pain experienced. Periapical radiographs were taken with customized Eggen holders for each patient to assess peri-implant bone levels at the second visit and at subsequent observation visits, according to Kotsakis *et al.* [45]. The customized bite registration material was disinfected and stored with the patient's study registration marked with a 5-digit ID code for use at subsequent evaluations evaluations. Blood specimen to determine soluble RANKL/OPG ratio (sRANKL/OPG) were drawn from the antecubical fossa in the second week.

3.4.4 Four and Eight week post-operative visits

Patients were seen at Four and Eight weeks post-surgeru for AE and SAE registration, self-report VAS (0-10 scale) and blood work for sRANKL/OPG.

3.4.5 Three month post implant placement visit

At three months following implant placement the implants were assessed for successful clinical integration according to the criteria established by Karoussis *et al*. [46]. In addition, Implant Stability Quotient (ISQ) values (Osstell ISQ ® Integration Diagnostics, Goteborg, Sweden) were evaluated. ISQ is an indirect measurement of the implant micro mobility, the metal rod (sensor) is connected to the inserted implant is subjected to 1N lateral load 10mm above the bone level, the sensor is subjected to magnetic pulses. High implant stability results in more sensor vibrations and higher ISQ values. ISQ values range from 0-100, 0-60 is considered to be low stability, 60-70 medium stability and 70+ high stability. The clinical significance of the ISQ values were described by *Nedir et al*, which found that an ISQ values of 54 predicted 100% osseointegration for immediately placed implants. The same group also recommended delayed loading when the ISQ values were <49 [53].

Karoussis *et al*. successful implant integration criteria consists of:

1. Absence of mobility
2. Absence of persistent subjective complaints (pain, foreign body sensation and or dysesthesia)
3. Probing depth (PD) ≤5mm
4. No PD≥5 and Bleeding on Probing (BOP) positive sites
5. Absence of continuous radiolucency around the implant
6. After the first year of service, the annual bone loss should not exceed 0.2 mm.

Successfully integrated implants were subsequently loaded with a fixed prostheses [46]. A periapical radiograph was taken with the previously fabricated customized Eggen holder and a blood draw for HbA1c level monitoring was also obtained at the 3 month post implant placement visit.

3.4.6 Restorative appointments

A digital impression of the integrated implants were taken using an intraoral scanner (Cerec Omnicam, Sirona Dental Systems LLC, Charlotte, NC, USA). The scan was transferred to an authorized straumann LTD laboratory (Minnesota Dental Lab, St. Paul, Minnesota, USA) for the fabrication of a titanium abutment and a full contour zirconia crown. The custom titanium abutment was tried in and a bite-wing radiograph was taken to confirm complete seating. The full contour zirconia crown was then inserted and adjusted until balanced proximal contacts were achieved with dental floss that snapped but did not shred. Shimstock occlusal paper was then slid over the implant crown to ensure it was held in place by the adjacent dentition. Lastly, all excursive contacts were eliminated. The custom titanium abutment was torqued to 35 N.cm with a Straumann torque wrench. A cotton pellet was inserted into the screw hole. The zirconia crown was then cemented with a thin layer of RelyX lutting cement (3M Espe Dental Products, St. Paul, MN, USA), for 3 minutes before all excess cement was removed. The proximal and occlusal contacts were rechecked and adjusted if necessary. A final bite-wing radiograph of the implant, abutment and crown was then taken.

3.4.7 Three and Six month post-loading visits

Patients were evaluated for clinical implant integration and early implant survival at three and six months post-loading using the criteria of Karoussis *et al.* [46] The examination included assessments of implant mobility, peri-implant inflammation, bone loss and pain evaluation. Periapical radiographs were taken with customized Eggen holders to assess implant crestal bone loss for each implant.

3.4.8 One and Three year post-loading follow-up visits

At 1-year and 3-years post-loading follow-up visits, periapical radiographs will be taken with customized Eggen holders for routine implant monitoring and will be evaluated to assess crestal bone loss for each implant. Peri-implant charting and HbA1c levels measurements will also be obtained.

3.5 Core histology

3.5.1 General fixation

The retrieved bone core was immediately placed in 10% formalin for overnight fixation. The core was washed three times in sterile saline to remove any residual formalin and placed in a secured vial with 70% ethanol before being transferred to the Masonic Cancer Center (St. Paul, Minnesota, USA) for additional processing. After decalcification in 10% Ethylenediaminetetraacetic acid (EDTA) for a few days the bone cores were dried with increasing percentages of ethanol washes (80%, 95%, 100%) for 35 minutes. Each ethanol wash was repeated twice. The bone cores were then transferred to xylene and later into hot paraffin using the Tissue Tek VIP (Tissue processor Sakura Finetek USA Inc, Torrance CA, USA) processor. Paraffin embedded bone cores were cut into 4-5 μm thicknesses using a microtome. The specimen was then fixed on a slide overnight at 37°C.

3.5.2 Hematoxylin and Eosin Staining & immunohistochemistry using Factor VIII

3.5.2.1 Preparation for staining

Bone core tissue sections were rehydrated in Xylene 3 times for 3 minutes each and later submerged in 100% ethanol twice for 1 minute each followed by 95% ethanol twice for 1 minute each and 70% ethanol once for 1 minute. The cut tissue sections were then washed under running tap water for 3 minutes for rehydration.

3.5.2.2 Hematoxylin staining

Hematoxylin (Harris Modified Hematoxylin with Acetic acid SH26-4D Fisher Scientific, Pittsburg, PA, USA) was applied for 3 minutes to the cut tissue sections prior to rinsing under running tap water for 3 minutes followed by a was with acid water (1.35 ml HCL + 900 ml distilled water) and finally running tap water again for 3 minutes. The core was placed in ammonium water (5 ml NH4OH + 175 ml Deuterium-Depleted water) for 15s and then in running tap water for 3 minutes to reach the desired intensity of the hematoxylin staining for cell nuclei labeling.

3.5.2.3 Eosin staining

Eosin (Surgipath, Buffalo Grove, IL, USA) was applied for 30 seconds for cytoplasm protein staining, followed by 95% ethanol twice for 30 seconds each and 100% ethanol twice for 30 seconds each. The cut tissue sections were submerged in Xylene 3 times for 30 seconds each. Finally, a drop of Permount (Fisher Scientific, Pittsburg, PA, USA) clearing mounting media was placed on the top of the sample and covered with a cover slip.

3.5.3 Immunohistochemistry

3.5.3.1 Preparation for staining

Bone core tissue sections were rehydrated in Xylene 3 times for 3 minutes each and later submerged in 100% ethanol twice for 1 minute each followed by 95% ethanol twice for 1 minute each and 70% ethanol once for 1 minute. The cut tissue sections were then washed under running tap water for 3 minutes for rehydration.

3.5.3.2 Indirect Immunohistochemistry

After paraffin removal and tissue rehydration, slides were placed in Tris-buffered saline (TBS-T) for 1 minute. Three percent hydrogen peroxide (H_2O_2) was used activity for 15 minutes at room temperature to block endogenous peroxidase. Samples were washed three times using TBS-T buffer at 2 minute intervals. In order to remove formalin fixation and enable the antigen to be more accessible for the primary antibody, enzymes were removed using pre-diluted Proteinase K for 5 minutes followed by 3 washes of TBS-T buffer. Undiluted protein block (Dako Protein Block Serum-Free X0909 Dako Corp. Carpinteria, CA, USA) was used to inhibit non-specific staining during Immunohistochemistry (IHC) for antigen detection. The protein block was applied to each slide for 15 minutes and the slides were then drained and wiped but not rinsed.

3.5.3.3 Primary antibody

Rabbit anti-human Von Willebrand Factor VIII (Dakot Corp, Carpinteria, CA, USA) was used with optimal dilution of 1:400 in Dako antibody diluent (Dako Corp. Capinteria, CA, USA) to label plasma glycoproteins and visualize blood vessels in the bone core tissue. For the negative control slides, the primary antibody was substituted with super sensitive rabbit negative control (BioGenex. Fremont, CA, USA). Slides were then incubated at room temperature for 60 minutes. Subsequently the slides then underwent three washes of TBS-T buffer at 2 minute intervals.

Antibodies were detected using undiluted Rabbit Envision+ (Dako Corp, Carpinteria, CA, USA) for 30 minutes at room temperature. The slides were then washed with TBS-T buffer at 2 minute intervals.

3.5.3.4 Chromogen Substrate

Sections were developed using a Diaminobenzidine (DAB) (Dako LSAB kit, K3468, Dakot Corp, Carpinteria, CA, USA). One drop of DAB was used per one ml of DAB dilution buffer. DAB was developed for 5 minutes and the slides were washed in running distilled water for 5 minutes.

3.5.3.5 Counterstaining

The slides were placed in Mayer's Hematoxylin solution (CS700, Dako Corp. Carpinteria, CA, USA) for 15 seconds for counterstaining and then washing in running distilled water for 5 minutes. Slides were then dehydrated through graded ethanol washes for 30 seconds; 70% once, 95% once and 100% three times. The slides were run through xylene for three times at 30 seconds each, a coverslip was placed over the slides before adding Permount for microscopic examination.

3.6 Bone core histology measurement

Due to a potential impact of the bone dust in the calculation of bone marrow areas and the estimation of vessel area and the vessel numbers, bone dust surface area was identified and calculated as a percentage of the total surface of the histological immunohistochemical section stained with Factor VIII. from each sample excluding the bone dust in the surrounding periphery. That bone dust percentage in each sample was used to calculate the bone marrow surface area, area of tissue, blood vessel surface area, percent of blood vessels surface area, number of blood vessels per sample and number of blood vessels per mm^2 per sample as depicted in Table 15.

3.7 Statistical analysis

In this preliminary analysis we compared the outcomes of different variables between a well controlled diabetic group (n=3) and a poorly controlled diabetic group (n=4) using two-sample t-test. The histological analysis in the well controlled diabetic group was performed on 2 samples, while the clinical analysis was performed on 3. The histological analysis and the clinical analysis were performed on all subjects (n=4) from the poorly controlled diabetic group. Each outcome was analyzed separately, including buccal and mesial ISQ values, insertion torques, mean bone marrow adipose surface area in mm^2, mean vital bone surface area in mm^2, mean blood vessel surface area in mm^2 and the mean number of blood vessels per mm^2 in each cohort. The ISQ values (Buccal/Mesial) were measured at baseline and at three month post implant placement. We compared the outcome at each time point and the change of outcome variable (Baseline – three month) between the two cohorts, separately. In addition, we compared the baseline and three month measurements for all patients using paired t-test. Significance was claimed for $p\text{-value} < 0.05$. The statistical test results for the ISQ intergroup comparison between the well controlled and the poorly controlled diabetic groups are shown in Table 7, with the mean and standard deviation (SD) for each outcome and for each cohort group. The statistical analysis for the histological differences between bone core

samples taken from each cohort are presented in Table 14.

Parameters were calculated using the two sample t-test for each cohort group, the mean adipose tissue surface area in mm^2, mean bone surface area in mm^2, mean blood vessel surface area in mm^2 and mean number of blood vessels per mm^2.

4. Results

4.1 Patient enrollment

Subject screening and enrollment is illustrated in Figure 2. A total of 32 patients were screened. However, 24 did not meet the inclusion criteria while 1 declined participation in the study. A total of 7 patients were enrolled into the study with 4 were allocated to the poorly controlled diabetic group and 3 to the well controlled diabetic group according to individual baseline HbA1c values. No patients were lost during follow-up or excluded from the analysis.

4.2 Patient demographics of the study population (n=7)

Enrolled patient demographics of the study population are presented in Table 1. All patients were older than 58 years of age at the time of enrollment. Enrolled patients consisted of five Caucasians and two African Americans. Three aprticipants were females and four were males. All of the females were enrolled into the poorly controlled group while three of the males were enrolled to the well controlled group and one to the poorly controlled group.

4.3 Implant placement appointment recorded information for each patient

During the implant placement appointment recorded information for each patient is shown in Table 2. All sites received a 4.1x10 implant. Bone core samples were obtained from all implant sites and ISQ values at implant placement ranged from 80-86 on the mesial implant surfaces and 79-85 on the buccal surfaces. The maximum insertion torque recorded was 60 N.cm while the minimum was 45 N.cm. No buccal or lingual dehiscence were present at the time of implant placement and hence none of the implants sites were grafted.

4.3.1 Seven days post operative follow up

Adverse events, change in medical history and VAS scores were obtained at the seven days post operative follow up appointment for all patients are shown in Table 3. Patient 001 (poorly controlled) presented with angular chielitis while Patient 003 (poorly controlled) reported a urinary infection that was managed by his physician. Two patients presented with post operative pain 1 week following surgery. Patient 001 (poorly controlled) self reported pain levels equal to 4 (moderate pain) while patient 007 (well controlled) reported pain levels equal to 2 (mild pain) using the Visual Analogue Scale (VAS) that was given to them.

4.3.2 Fourteen days post operative follow up for all patients

Adverse events, change in medical history and VAS scores at the fourteen days post operative follow up appointment for all patients are depicted in Table 4. No further adverse reactions or changes in medical history were reported by the patients. All participants expressed 0 out of 10 pain levels using the VAS system.

4.3.3 Implant related outcomes at the three months post implant placement appointment

Implant related outcomes at the 3 months post implant placement appointment are illustrated in table 5. None of the patients had any adverse reactions associated with the implant placement. Patient 004 (well controlled) was instructed by his physician to stop his anti-diabetic medication, which resulted with an increased HbA1c (11%). Overall, the mesial ISQ values ranged from 84-87 while the buccal ISQ values ranged from 84-88. None of the implants presented with clinically visible mobility. At the three months time period with respect to the well controlled diabetic patients, the HbA1c values for patient 002 stayed within the well controlled group's inclusion criteria ($5.8\% < HbA1c \geq 7\%$) while patients 004 and 007 had elevated HbA1c% levels with 11.0% and 7.6% respectively.

4.3.4 Three months post crown delivery appointment

Findings for adverse reactions, change in medical history, peri-implant probing depths and implant mobility at the three months post crown delivery are shown in Table 6. Four of the patients, exhibited peri-implant probing depths were 1-3 mm. However, for patient 005 (poorly controlled) there was a single surface (Mesio-Buccal) that was positive for bleeding on probing with the overall probing depths ranged between 2-4 mm. None of the implants presented with any clinically diagnosed mobility at this time frame. All patients received a cement retained crown, yet information about patients 006 and 007 wasn't included due to the fact that three months hadn't passed since the crown delivery.

4.3.5 ISQ intergroup comparison

ISQ intergroup comparison between the well controlled and the poorly controlled diabetic groups is shown in Table 7. Both study groups achieved favorable baseline (implant placement) and three months post placement mean ISQ values and insertion torques. The mean baseline buccal ISQ values for the well controlled group was 84.666 while 82.000 was recorded in the poorly controlled group. At three months post implant placement slightly higher mean ISQ values were recorded with 85.666 for the well controlled group and 85.250 for the poorly controlled group. The mean change in buccal ISQ values between baseline and at the three months appointment was 1.000 for the well controlled group and 3.250 for the poorly controlled group.

The mean baseline mesial ISQ values for the well controlled group was 85.000 while for the poorly controlled group a mean ISQ of 83.250 was recorded. At the three months post operative appointment the mean mesial ISQ values for the well controlled group was 85.333 while 85.500 for the poorly controlled group was recorded. The mean change in mesial ISQ values between baseline and the three months appointment was 0.333 for the well controlled group and 2.250 for the poorly controlled group.

In both groups the implants were inserted with high mean insertion torques, 56.666 N.cm for the well controlled group and 51.250 N.cm for the poorly

controlled group. There were no statistically significant differences ($p>0.05$) between the two study groups for any recorded parameter.

4.4 Histology

4.4.1 Hematoxylin and Eosin (H&E) staining of bone core samples from a representative well controlled diabetic patient and from a representative poorly controlled diabetic patient

Figure 3A depicts a sectioned bone core that has been stained with H&E and viewed at 20x magnification to represent a well controlled diabetic patient. The cut bone core section includes the bone surface area, the bone marrow and the sawdust (which is a consequence of bone harvesting with the trephine drill). This surface area was used for evaluation of the total cut bone core sectioned surface area for each patient in the well controlled diabetic group. Surface bone area with the bone marrow and sawdust removed from a well controlled diabetic patient is presented in Figure 3B. This sample was used for determination of the total bone surface area in cut sections harvested from well controlled diabetic patients. Surface bone marrow area from a well controlled diabetic representative is shown in Figure 3C. The cut sections were divided to 4 equal parts. These divided cut sections were used for the calculation of bone marrow surface area depicted as encircled in yellow color.

Figure 4A represents the cut bone core section stained with H&E at a 20x magnification from a poorly controlled diabetic patient are presented in Figure

4A.

Similarly, to Figure 3A, the entire bone core cut section includes the bone surface area, bone marrow and sawdust. This surface area was used for evaluation of the total cut bone core sectioned surface area for each patient in the well controlled diabetic group. Surface bone area with the bone marrow and sawdust removed from a poorly controlled diabetic patient is presented in Figure 4B. Determination of the total bone surface area in the cut sections from poorly controlled diabetics was done by removing the sawdust and areas identified as bone marrow. Surface bone marrow area from a poorly controlled diabetic is shown in Figure 4C. Again, the cut sections were divided to 4 equal parts. The divided cut sections were used for the calculation of bone marrow surface area as encircled in yellow color. Bone core section stained with Hematoxylin and Eosin from the well-controlled diabetic and poorly controlled diabetic groups are depicted in Figures 11 and 12.

4.4.2 Factor VIII staining for blood vessel detection from a representative well controlled diabetic patient and from a representative poorly controlled diabetic patient

The bone core cut sections also underwent through immunohistochemistry with Factor VIII to identify blood vessels within the cut sections. A bone core cut section from a representative well controlled diabetic patient using immunohistochemistry with Factor VIII is depicted in Figure 5. Areas that stained positively with Factor VIII and exhibited a lumen like area were considered blood vessels and were encircled in yellow color.

Similarly, bone core cut section from a representative poorly controlled diabetic patient using immunohistochemistry with Factor VIII is illustrated in Figure 6. Positively stained surfaces that exhibited a lumen like area were again considered blood vessels and encircled in yellow color. The number of blood vessels and blood vessel total surface area were calculated using the yellow encircled areas from each bone core cut section using 40x magnification. Immunohistochemistry using Factor VIII from the well controlled and poorly controlled diabetic samples are depicted in Figures 13 and 14.

4.4.3 Bone core analysis

4.4.4 Bone Surface Area in mm^2

For the calculation of the Total bone surface area in each sample, was calculated subtracting values for bone marrow and sawdust. Hematoxylin & Eosin staining analysis of total bone surface area in mm^2 is presented in Table 8. These samples were divided into 4 equal parts that were analyzed individually and summed for determination of whole bone surface area in mm^2 from each sample. The total bone surface area from the well controlled group ranged from 7.33 mm^2 to 10.21 mm^2 while in the poorly controlled group it ranged from 3.58 mm^2 to 12.32 mm^2.

4.4.5 Area of Bone Marrow in mm^2

Bone marrow surface area was identified using the bone core samples stained with Hematoxylin & Eosin. Areas that were recognized as bone and sawdust were not included in the bone marrow surface area calculation. All areas that included bone marrow were encircled in yellow and the total surface area was calculated in mm^2. Hematoxylin & Eosin staining analysis of total bone marrow surface in mm^2 is shown in Table 9. Briefly, the 2 well controlled group samples were relatively similar with 0.77 mm^2 and 0.79 mm^2 of total bone marrow surface area. The poorly controlled group showed higher variability with sample 006 having 0.00 mm^2 bone marrow surface area compared to sample 001 with 4.57 mm^2. When looking at the cancellous 25% area, it is noteworthy that no area of fatty bone marrow in mm^2 (0.00) for samples 003, 005, 006 (poorly controlled) and for sample 004 (well controlled) was found. Sample 001 was shown to have a larger bone marrow surface area in mm^2 than all of the other samples combined regardless of their group allocation.

4.4.6 Total live tissue area in mm²

Factor VIII histomorphometrical analysis of total live tissue area in mm² is presented in Table 10. Using 40x magnification, the total area of live tissue in mm² was calculated including the bone marrow, bone and blood vessels surface area. Sawdust was removed for this analysis. The results of this histomorphometrical analysis is illustrated in Table 10. The well controlled group total surface area in mm² ranged from 12.04 to 13.22, while in the poorly controlled group it ranged from 4.79 mm² to 13.34 mm².

4.4.7 Total Blood Vessel Surface Area in mm²

Slides stained with the immunohistochemistry technique for endothelial cells detection (Factor VIII) were examined under 40x magnification. Areas that were stained positively with Factor VIII and presented with a lumen like area were considered as blood vessels and encircled in yellow. Factor VIII histomorphometrical analysis of total blood vessel surface area in mm² is shown in Table 11. In the well controlled diabetic group, sample 002 had 0.157 mm² blood vessel surface area while sample 004 had 0.632 mm². The poorly controlled diabetic group total blood vessel surface area ranged from 0.104 mm² to 0.290 mm². Surprisingly, in sample 005 (poorly controlled) blood vessels were

identified only in the top cortical quadrant.

4.4.8 Number of Blood Vessels per sample & percent of blood vessel total surface area

The number of blood vessels per sample and the percent of blood vessel total surface area for each sample are presented in Table 12. In the well controlled diabetic group, sample 002 had 32 blood vessels per sample, while sample 004 had 151. For the poorly controlled group the number of blood vessels per sample ranged from 35 to 56. For the whole area of blood vessel in mm^2, the well controlled diabetic group ranged from 0.157 mm^2 to 0.632 mm^2 while the poorly controlled diabetic group ranged from 0.117 mm^2 to 0.290 mm^2. Also, the percent of total blood vessel surface area is shown in Table 12. The percent of total blood vessel surface area was calculated by dividing the whole area of blood vessels mm^2 by the whole live tissue area in mm^2 multiplying by a hundred for each sample. The well controlled diabetic group ranged from 1.308 % to 4.785 % of percent blood vessels area while the poorly controlled diabetic group ranged from 0.779% to 3.478%.

4.4.9 Number of Blood Vessels per mm² for each sample

The number of blood vessels per mm² for each sample is presented in Table 13. For calculation of the number of blood vessels per mm² the blood vessels were manually counted and divided by the total bone surface area in mm² for each specimen. The well controlled group ranged from 3.134 blood vessels per mm² to 20.600, while in the poorly controlled group the results ranged from 2.840 to 13.128 blood vessels per mm².

4.4.9.1 Histological differences between the bone core samples that were taken from the well controlled diabetic group and the poorly controlled diabetic group.

Histological differences between the bone core samples that were taken from the well controlled diabetic group and the poorly controlled diabetic group are presented in Table 14. The analyzed histological parameters include the mean Adipose Tissue/Sample mm², mean Bone Surface Area/ Sample mm², Mean Blood vessel surface area mm² and Mean Number of Blood Vessels per mm². Amongst the compared parameters, mean Adipose Tissue/Sample mm² was shown to be 0.780 mm² in the well controlled diabetic group compared to 1.350 mm² in the poorly controlled diabetic group.

The standard deviation in the well controlled diabetic group was low (0.014) while the poorly controlled diabetic group had a higher standard deviation (2.169). The difference between the two groups was not found to be statistically significant ($p>0.05$); but a trend was still observed. The poorly controlled diabetic group demonstrated almost twice the amount of total adipose tissue surface area of 1.35 mm^2 as compared to 0.78 mm^2 in the well controlled diabetic group as illustrated.

Another compared parameter was the mean Bone Surface Area/Sample mm^2. The mean bone surface area in mm^2 of cut tissue samples taken from the well controlled diabetic group and the poorly controlled diabetic group can be seen in Table 14. The well controlled diabetic group presented with 8.770 mm^2 (SD=2.036) while the poorly controlled diabetic group had 7.220 mm^2 (SD=3.666). The difference between the two groups was not found to be statistically significant ($p>0.05$).

The mean blood vessel surface area in mm^2 calculated from bone core samples taken from well controlled and poorly controlled diabetic patients is shown in Table 14. The mean Area Blood Vessels/Sample mm^2 in each cohort was determined for the well controlled diabetic group to be 0.395 mm^2 (SD=0.335) compared to 0.159 mm^2 (0.088) in the poorly controlled diabetic group. Again, the difference was not found to be statistically significant ($p>0.05$), yet a trend can be observed with more than double the Area of Blood Vessels/Sample mm^2

in the well controlled diabetic group than the poorly controlled diabetic group.

The mean number of blood vessels per mm² was calculated from bone core samples taken from well controlled and poorly controlled diabetic patients is shown in Table 14. The well controlled group had 11.867 blood vessels per mm² compared with 8.229 per mm² in the poorly controlled group. The standard deviation in the well controlled diabetic group was 12.350 while the poorly controlled diabetic group had a relatively smaller standard deviation of 4.212. The difference between the two cohorts was not found to be statistically significant with $p>0.05$.

The mean bone marrow tissue surface area in mm² was calculated from cut bone core samples taken from well controlled (0.700 mm²) and poorly controlled (1.06 mm²) diabetic groups are illustrated in Figure 7. The mean Bone Surface Area/Sample mm² calculated from bone core samples taken from well controlled (8.770) and poorly controlled diabetic patients (7.220) is shown in Figure 8. Mean blood vessel surface area of each cohort in mm² calculated from bone core samples taken from well controlled and poorly controlled diabetic patients is presented in Figure 9. The mean number of blood vessels per mm² in each cohort group calculated from bone core samples taken from well controlled (11.85) and poorly controlled (8.17) diabetic patients is depicted in Figure 10.

5. Discussion

It is well established that Type II DM may result in accumulation of AGEs, which compromises the bone matrix properties due to defective collagen production. The AGEs have been found to alter osteoblastic proliferation and increase osteoclastic bone resorption [47]. In a recent review, it was shown that Type II DM mice presented with a significantly lower Bone to Implant Contact when compared with healthy mice. It was also found that the majority of alterations were found to be in the cortical bone area while the cancellous bone was less affected by these alterations [48]. In a more recent study, whereby Type II DM patients who underwent limb amputation due to critical ischemia, it was exhibited that Type II DM bone marrow alterations that resulted in decreased hematopoietic tissue [10,49]. The authors suggest that this could lead to an increase in bone marrow adiposity and that these changes were found to be inversely associated with decreased distal femur bone volumes in animals. An earlier histomorophometric study found a direct association between increased bone marrow adiposity and decreased osteoblastic activity in 51 human iliac crest biopsies [50]. Decreased osteoblastic activity had been previously associated with decreased bone formation and mineral apposition [10,11,22]. Spinetti *et al*. used histomorphometric evaluation of bone marrow adiposity in patients that underwent hip replacement [49]. They concluded that the adipose

tissue fraction difference between the healthy control group and the Type II DM group was statistically significant ($p<0.001$).

In this study, we also attempted to compare the adipose tissue surface area in mm^2 of well controlled and in poorly controlled Type II DM patients. However, our findings didn't reach statistical significance with a relatively small sample size of evaluated subjects. Nevertheless, a trend was present with almost double adipose surface area in the poorly controlled group compared to the well controlled group. A large standard deviation (2.169) in the poorly controlled group was observed which was not seen in the well controlled group (0.014). The higher standard deviation must be interpreted carefully due to the small sample size in this study.

Our investigation also evaluated blood vessel density. Stabley and co-investigators found that Type II DM rats might have altered blood flow in their marrow when compared to healthy rats [51]. The investigators concluded that these alterations may eventually lead to osteopenia. Teraa *et al.* noted in a human histology study of patients with critical limb ischemia, that the blood vessel density was lower in patients with Type II DM (32.3 microvessels/mm^2) compared to healthy controls (40.2 microvessels/mm^2), the difference between the two groups was found to be statistically significant ($p=0.01$) [52]. Spinetti compared bone biopsies for blood vessel density in patients with Type II DM with non-diabetic healthy controls undergoing a full hip replacement procedure [35].

Histomorphometry using CD31 and Factor VIII were performed on the hip bone samples. A total of 49 patients were enrolled into the control non-diabetic group and 10 patients into the Type II DM group. It was found that Type II DM patients presented with 11.3 capillaries per mm² while healthy individuals presented with 25.3 capillaries per mm². The difference was statistically significant with a relatively small standard deviation (2.4 and 3.1, respectively). The authors concluded that a statistically significant decrease of capillary density was observed and that the dependent microvascular variables included diabetes mellitus duration and fasting glucose levels. In this study, the difference between the blood vessel density in the well controlled and poorly controlled Type II DM groups was not found to be statistically significant ($p>0.05$). Nevertheless, a trend can be observed with 11.867 blood vessels per mm² in the well controlled diabetic group and 8.229 per mm² in the poorly controlled diabetic group. Both the Teraa *et al.* [34] and Spinetti *et al.* [35] studies have presented with a significantly higher number of blood vessels per mm² when compared to the results of this study. The differences might be attributed to the inherent characteristics of the mandibular bone when compared to long bones (hip/limbs) and, perhaps as elucidated by Spinetti, by the diabetic duration and overall fasting glucose levels.

Regardless of the quantitative difference between the two diabetic groups, all implants were successfully placed with a high insertion torque and ISQ values. A healing abutment was secured in a non-submerged healing site allowing for a standardized implant placement protocol. At the 7 days post operative appointment minimal adverse reactions were recorded with only angular cheilitis and a urinary infection affecting two patients. Minimal post operative pain was noted according to VAS scale was communicated to the investigators. At the 14 days post-operative appointment, no adverse reactions were recorded and no post operative pain was reported by the patients. It is important to point out that one patient was instructed by their physician to stop taking their diabetes medication, consequently their HbA1c levels increased up to 11% at the 3 months post operative appointment. Surprisingly, the change in HbA1c didn't affect implant integration or peri-implant bone levels and probing depths. None of the patients presented with positive bleeding on probing in the peri-implant gingival sulcus except for patient 005 who was in the poorly controlled diabetic group.

Because of the limited number of patients evaluated, the reported results should be interpreted with caution. Significant efforts were taken to initiate this investigation and enroll the initial patients by the investigators. With continued enrollment of a greater number of patients the resulting data may validate our conclusions.

6. Conclusions

Within the limitation of this study it can be concluded that:

1. The well controlled diabetic group (5.8%<HbA1c≥7%) presented with a mean of 11.867
   blood vessels per mm² as compared with 8.229 per mm² in the poorly controlled diabetic
   (7.5% <HbA1c>10%) group.

2. When comparing the mean bone marrow surface area it was found that the poorly
   controlled diabetic group (7.5% <HbA1c>10%) presented with 1.06 mm² compared to
   0.70 mm² in the well controlled diabetic group (5.8%<HbA1c≥7%).

3. Within the first 6 months after implant placement it can be concluded that hydrophilic
   TiZr implant surfaces (Roxolid®) yielded similar early implant stability,
   survival and success rates in poorly and well controlled Type II DM individuals

whereby all implants were successful according to the criteria of Karoussis *et al* [20].

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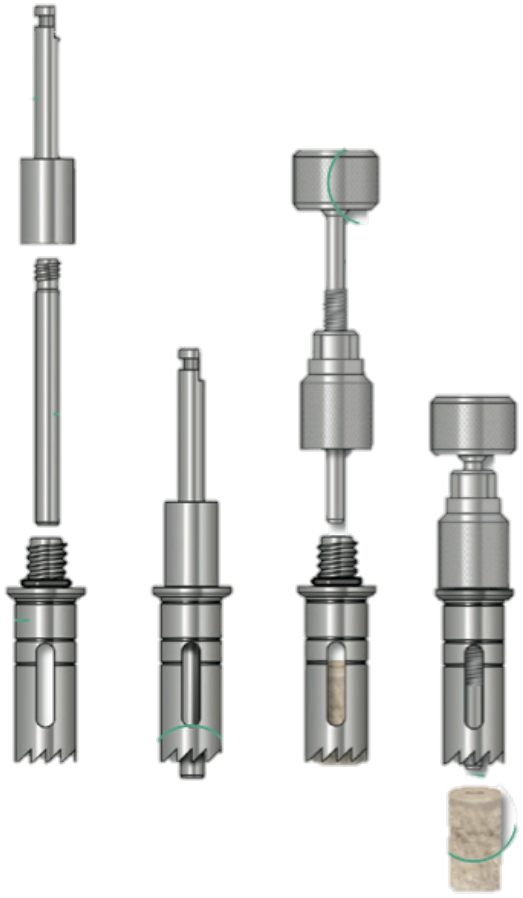
Appendix

Figures

Figure 1. ACE trephine system assembly for bone harvesting.



1A. 1B. 1C. 1D.

Figure 1A. Unassembled ACE trephine system. **Figure 1B.** Assembly of the three piece ACE trephine system for bone core retrieval. **Figure 1C.** After retrieval of the bone core sample a fourth piece is introduced to gently push the bone core out of the trephine. **Figure 1D.** The bone core sample removed from the three piece ACE trephine system.

Figure 2. Patient screening and enrollment

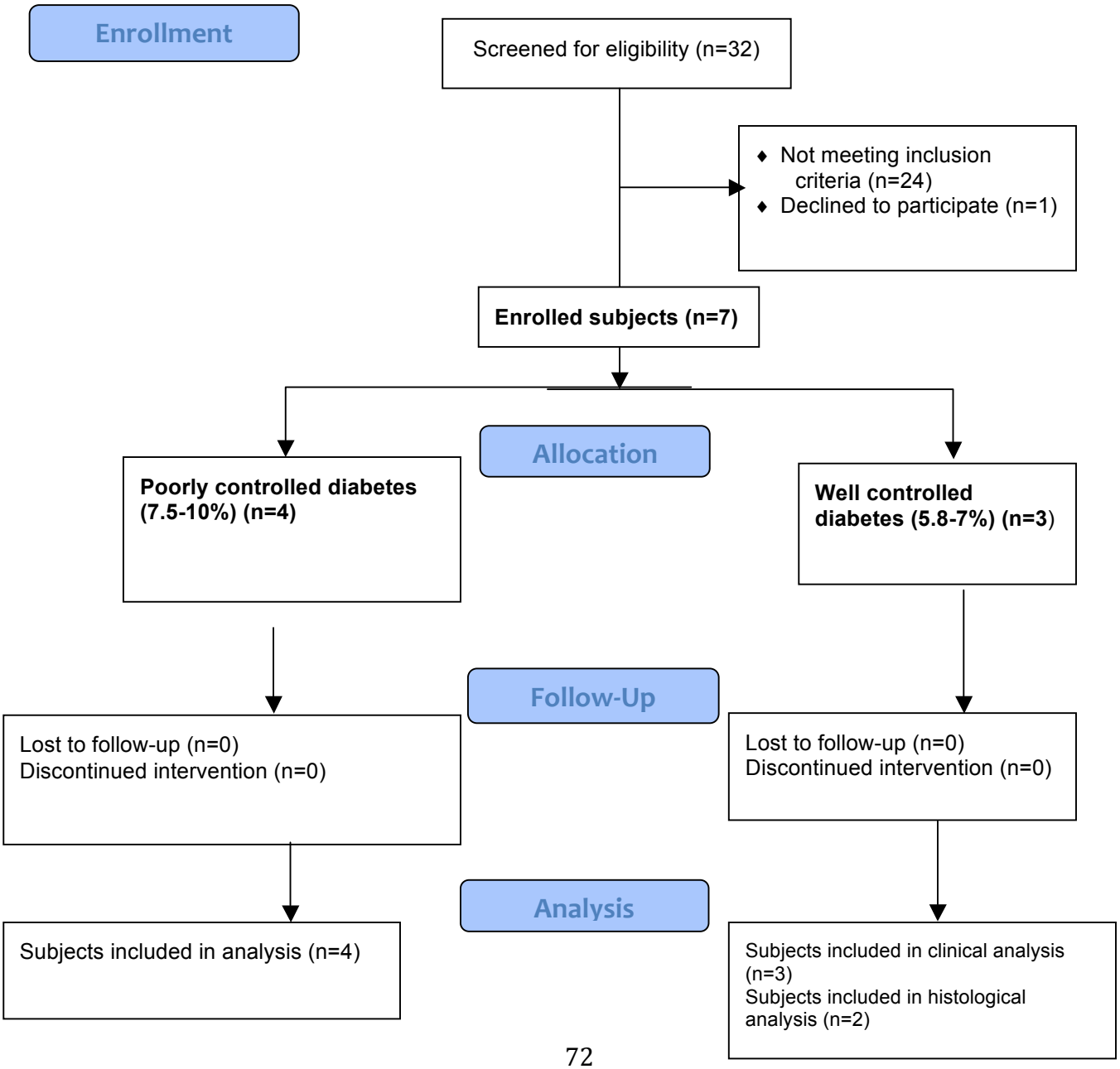
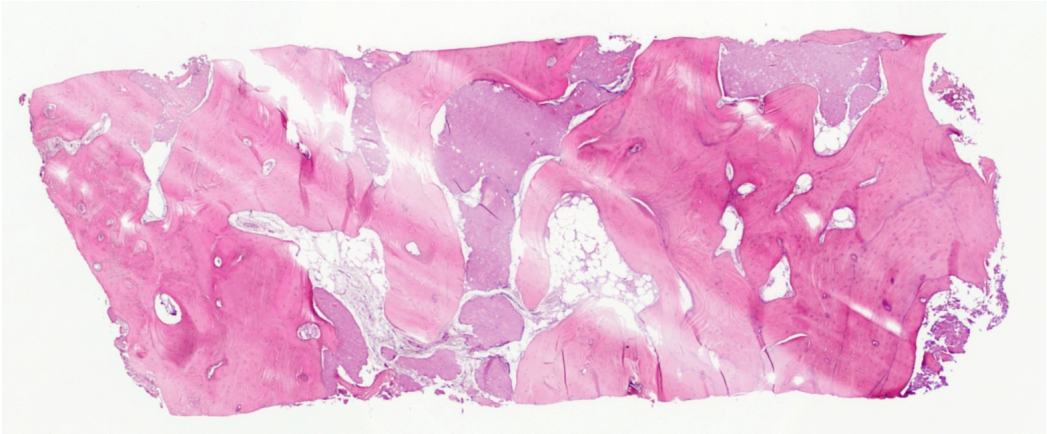


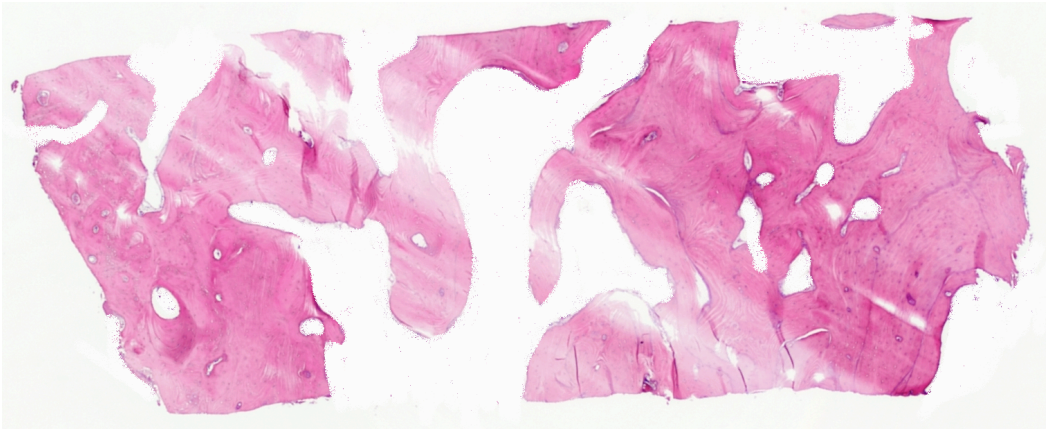
Figure 3. Stained bone core section stained with Hematoxylin and Eosin from a well controlled diabetic patient (002) in 20x magnification.

Figure 3A Represents the total bone core (including bone surface area, bone marrow and sawdust). **Figure 3B** Bone marrow and sawdust are removed and only the bone surface area is present. **Figure 3C** The bone marrow surface area encircled in yellow.

3A



3B



3C

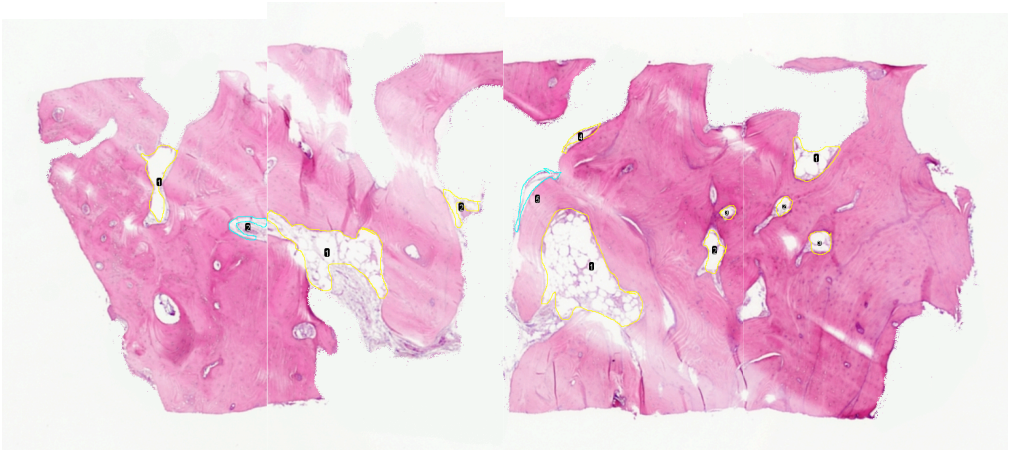
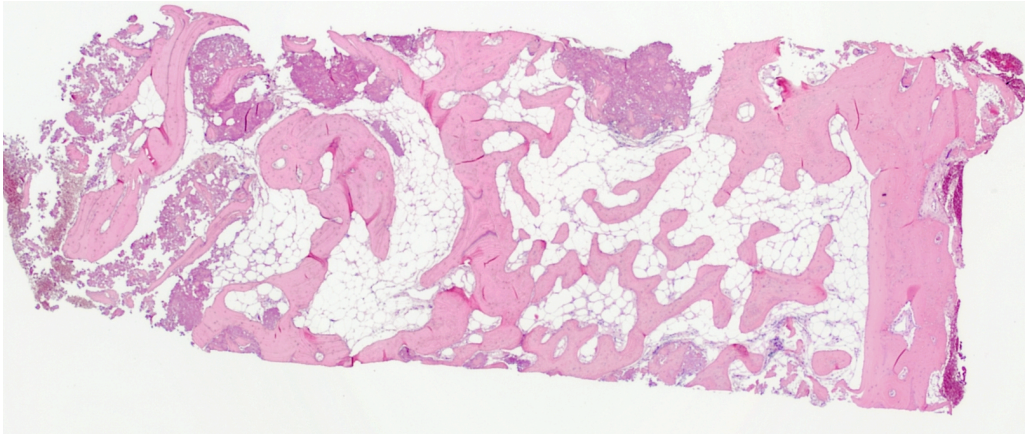


Figure 4. Stained bone core section stained with Hematoxylin and Eosin from a poorly controlled diabetic patient (002) in 20x magnification. **Figure 4A** represents the total bone core (including bone surface area, bone marrow and sawdust). **Figure 4B** shows the bone surface area only (bone marrow and the sawdust were removed). **Figure 4C** illustrates the bone marrow surface area (encircled in yellow).

4A



4B

4C

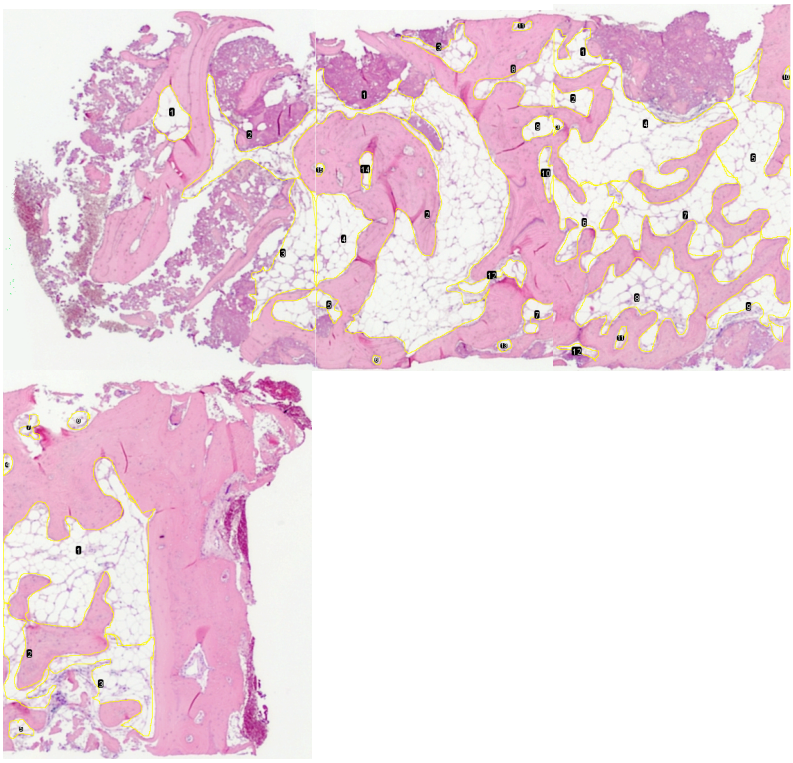


Figure 5. Using immunohistochemistry (Factor VIII) to specifically identify endothelial cells from sample 002 (Well controlled diabetic group). Positively stained cells that present with a lumen like areas were identified as blood vessels and encircled in yellow.

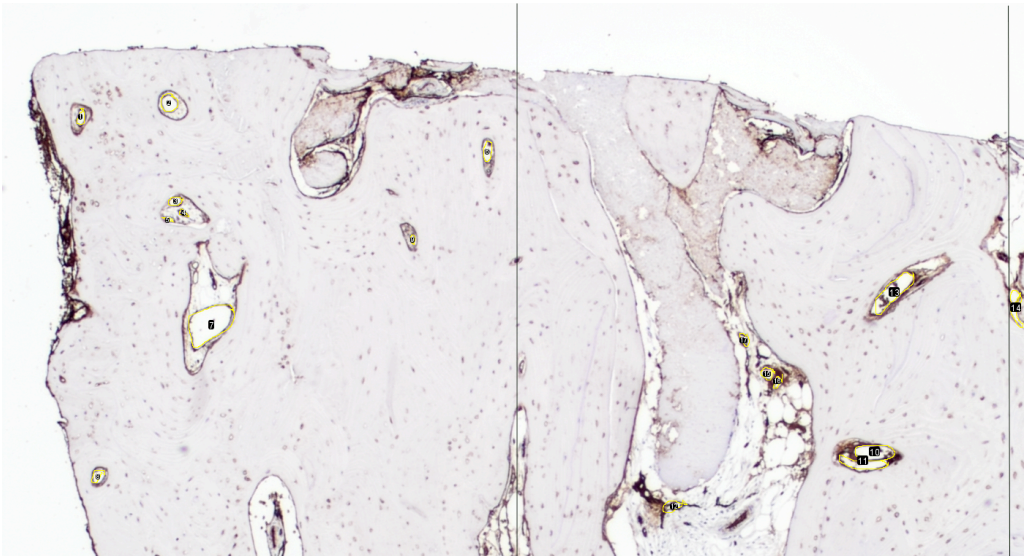


Figure 6. Using immunohistochemistry (Factor VIII) to specifically identify endothelial cells from sample 001 (Poorly controlled diabetic group). Positively stained cells that present with a lumen like areas were identified as blood vessels and encircled in yellow.

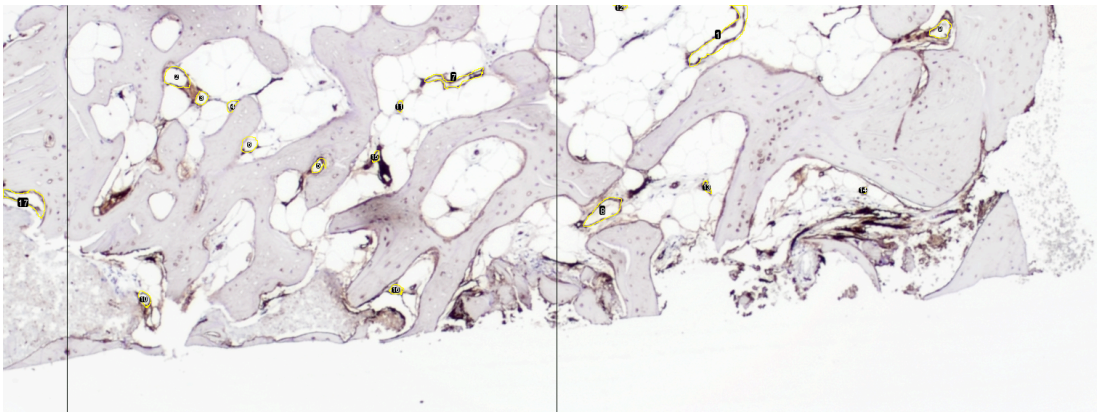


Figure 7. Mean bone marrow surface area in mm² calculated from bone core samples

taken from well controlled and poorly controlled diabetic patients.

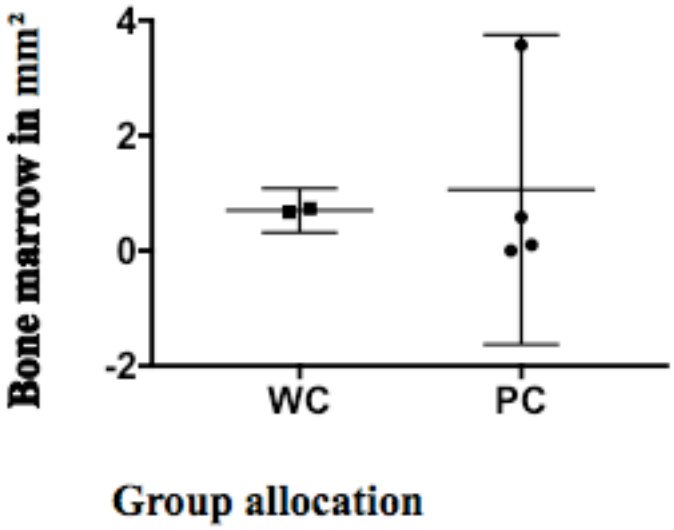


Figure 8. Mean bone surface area in mm² calculated from bone core samples taken from

well controlled and poorly controlled diabetic patients.

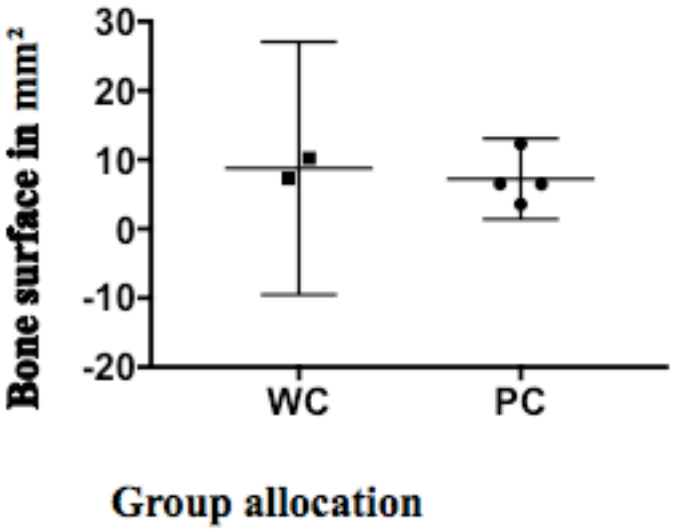


Figure 9. Mean blood vessel surface area of each cohort in mm² calculated from bone core samples taken from well controlled and poorly controlled diabetic patients.

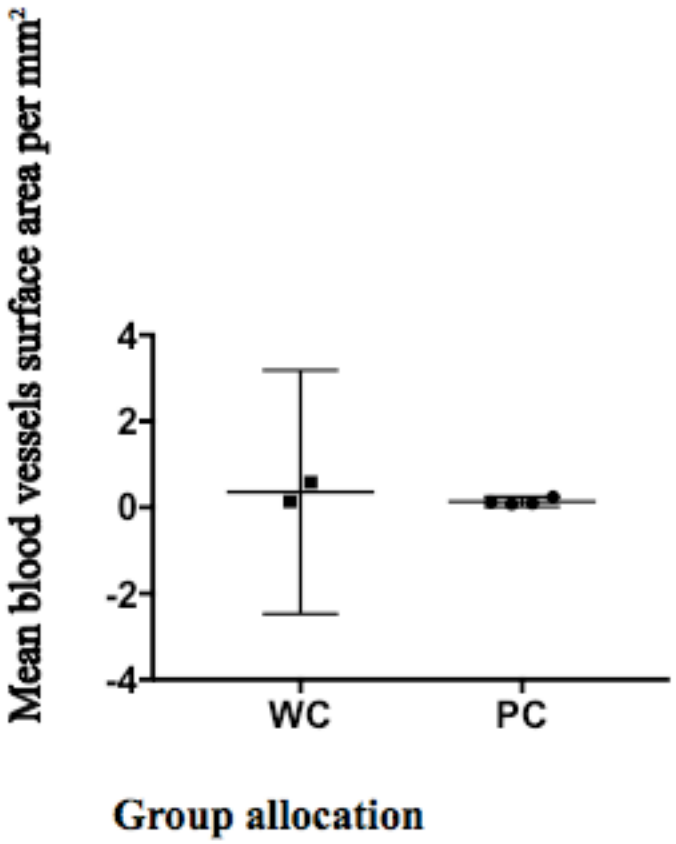


Figure 10. Mean number of blood vessel per mm² calculated from bone core samples

taken from well controlled and poorly controlled diabetic patients.

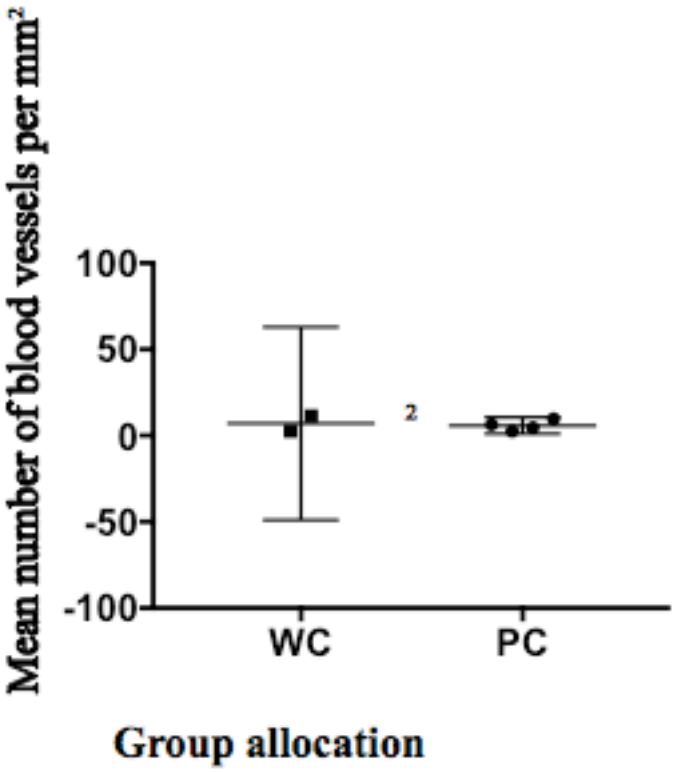
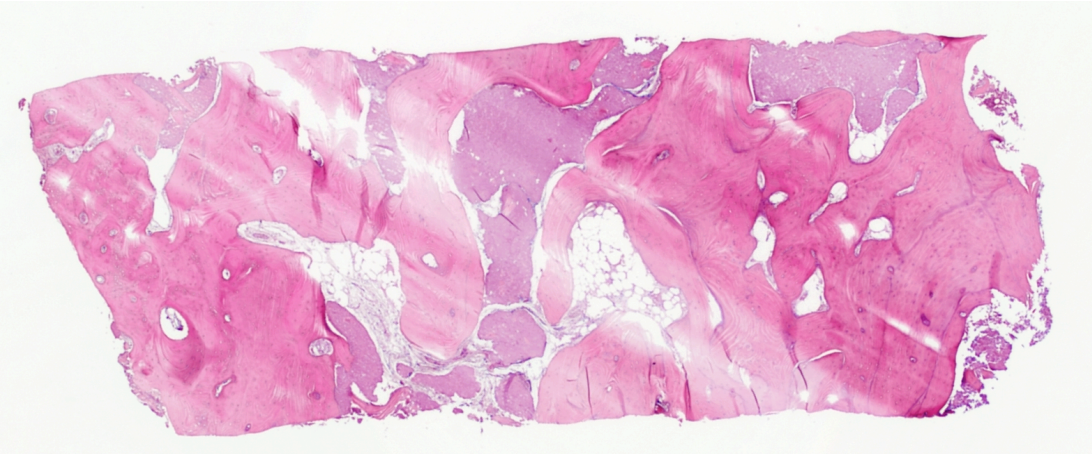


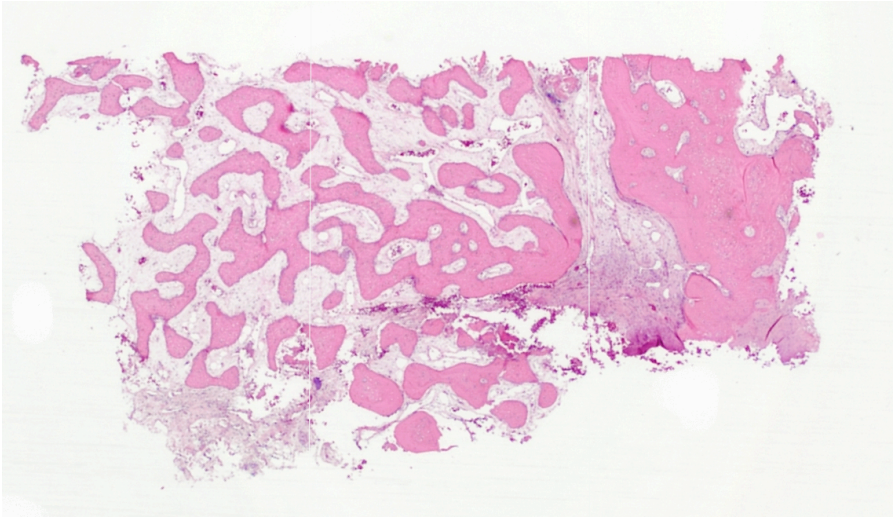
Figure 11. Stained bone core section stained with Hematoxylin and Eosin from the well-controlled diabetic group in 20x magnification.

002



004 A and 004 B (Sample fractured during harvesting)

A.



B.

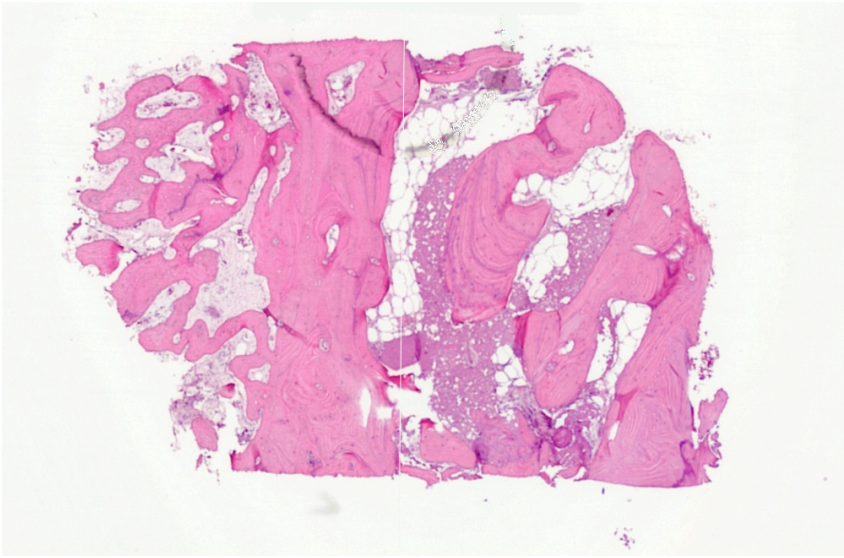
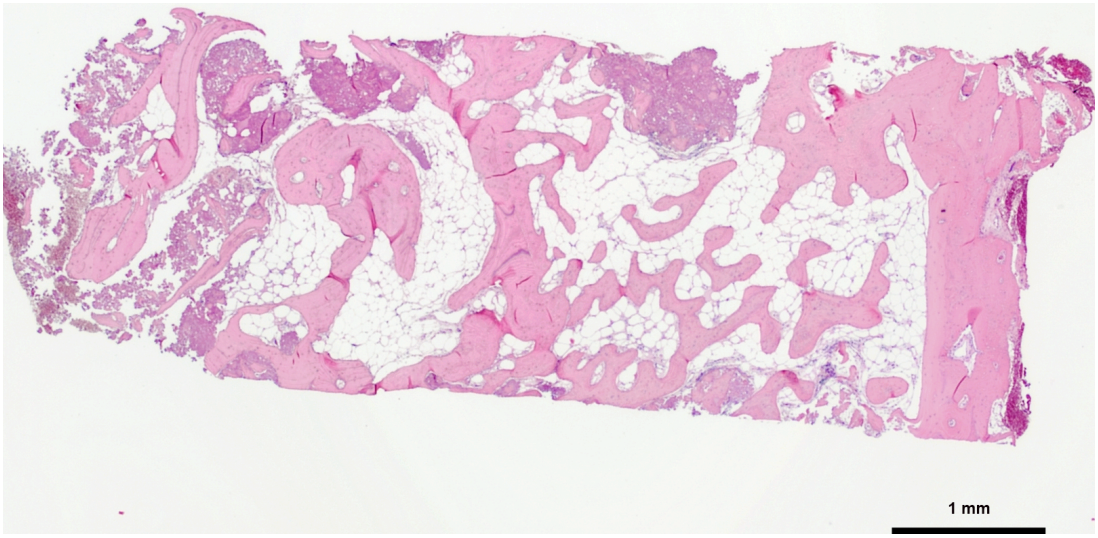
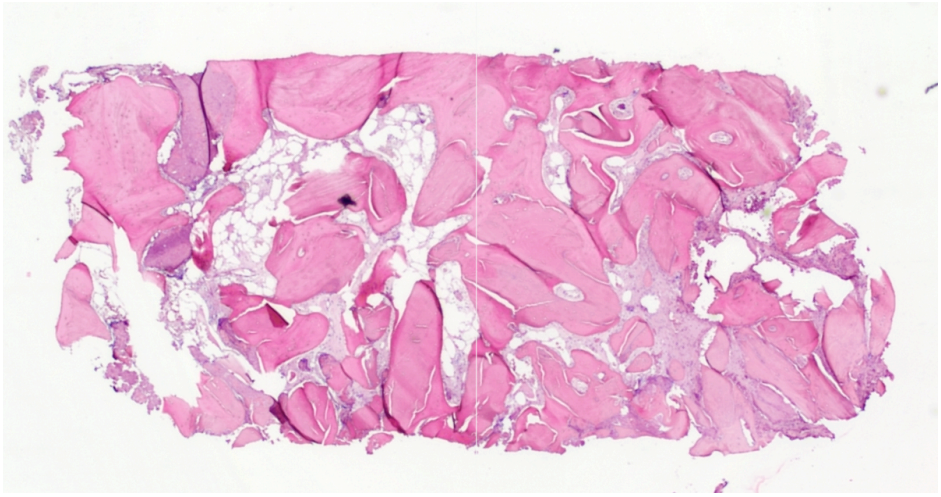


Figure 12. Stained bone core section stained with Hematoxylin and Eosin from the poorly-controlled diabetic group in 20x magnification.

001



003



005

006

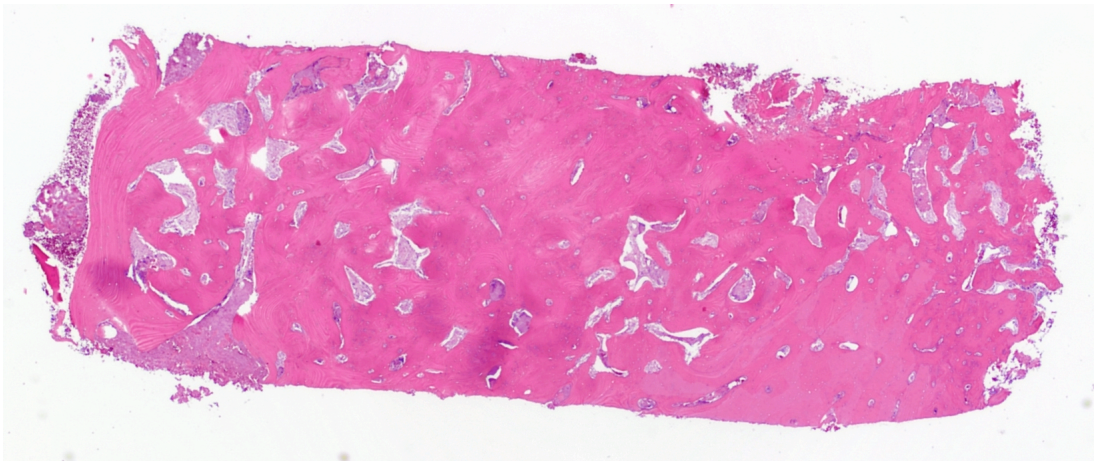
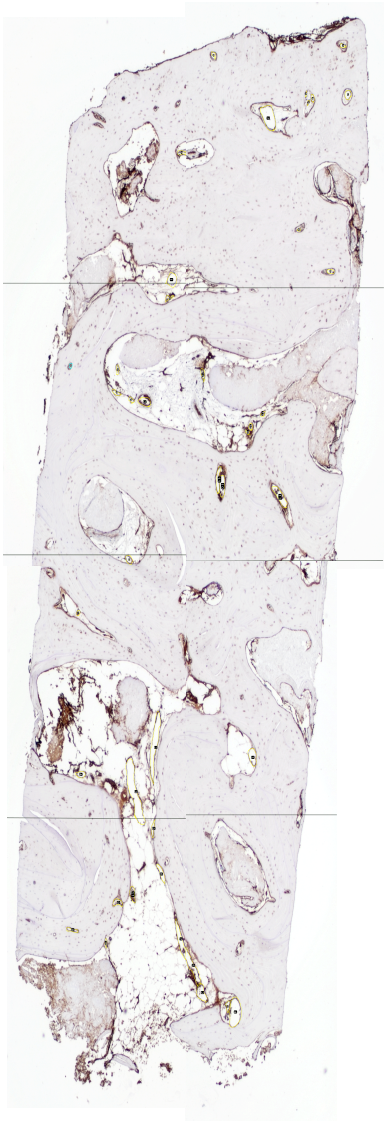


Figure 13. Immunohistochemistry using Factor VIII from the well controlled diabetic

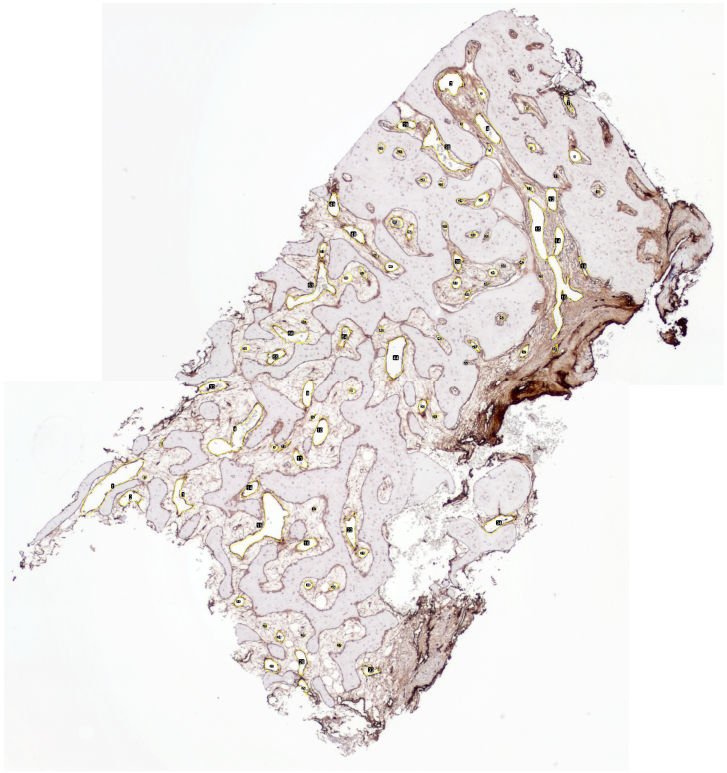
group 40x magnification.

002



004

004 A



004 B

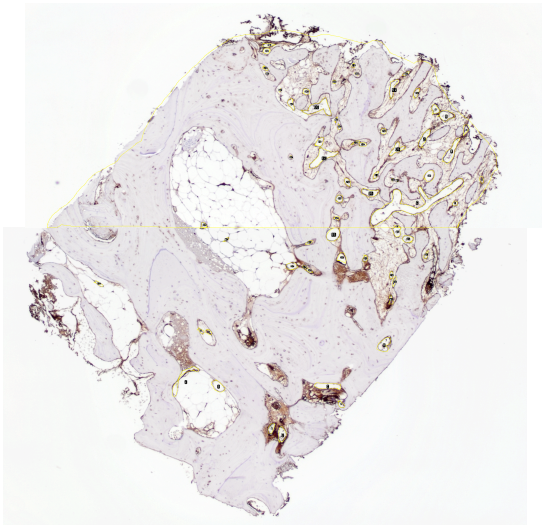
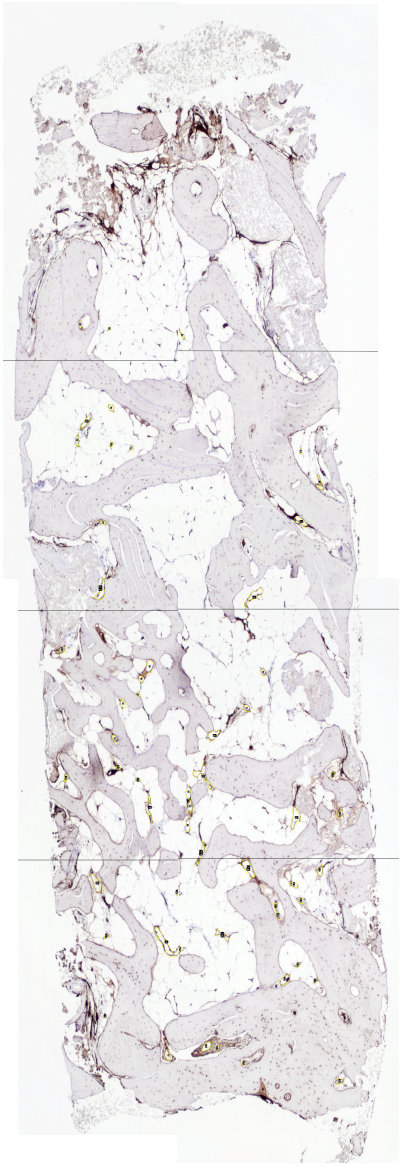


Figure 14. Immunohistochemistry using Factor VIII from the poorly controlled diabetic

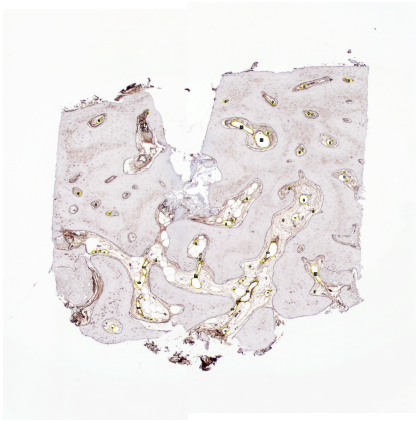
group 40x magnification.

001



003

005



006

Tables

Table 1. Patient demographics of the study population (n=7).

Patient I	Baseline HbA1c	Cohort allocation	Age	Implant site	Ethnicity*	Race	Gender
004	6.9%	Well controlled	68	20	NHL	Caucasian	Male
002	6.3%	Well controlled	58	30	NHL	Caucasian	Male
007	7.0%	Well Controlled	64	29	NHL	African American	Male
003	9.2%	Poorly controlled	68	20	NHL	Caucasian	Male
001	8.1%	Poorly controlled	60	19	NHL	Caucasian	Female
005	8.5%	Poorly controlled	64	30	NHL	Caucasian	Female
006	8.7%	Poorly controlled	72	28	NHL	African American	Female
007	7.0%	Well Controlled	64	29	NHL	African American	Male

* NHL: Non-Hispanic or Latin

Table 2. Implant placement appointment recorded information.

Patient ID and study group•	**Implant placed**	**Bone core obtained**	**ISQ values (Triplicate average)**	**Maximum insertion torque (N.cm)**	**Buccal dehiscence present (Yes/No)**	**Lingual dehiscence present (Yes/No)**	**Implant site grafted (Yes/No)**
004 WC	4.1x10	Yes	Mesial 84 Buccal 84	60	No	No	No
002 WC	4.1x10	Yes	Mesial 86 Buccal 85	50	No	No	No
007 WC	4.1x10	Yes	Mesial 85 Buccal 85	60	No	No	No
003 PC	4.1x10	Yes	Mesial 80 Buccal 82	50	No	No	No
001 PC	4.1x10	Yes	Mesial 85 Buccal 85	50	No	No	No
005 PC	4.1x10	Yes	Mesial 82 Buccal 82	45	No	No	No
006 PC	4.1x10	Yes	Mesial 86 Buccal 79	60	No	No	No

• WC- Well controlled group; PC –Poorly controlled group

Table 3. Adverse events, change in medical history and VAS scores at the seven days post operative follow up appointment.

Patient ID and study group•	**Adverse events/ Change in medical history**	**VAS⌖ score (0-10)**
004 WC	No	0
002 WC	No	0
007 WC	No	2
003 PC	Urinary infection	0
001 PC	Angular chielitis	4
005 PC	No	0
006 PC	No	0

• WC- Well controlled group; PC- Poorly controlled group

⌖VAS-Visual Analogue scores

Table 4. Adverse events, change in medical history and VAS scores at the fourteen days post operative follow up appointment for all patients.

Patient ID and study group•	**Adverse events/ Change in medical history**	**VAS⌖ score (0-10)**
004 WC	No	0
002 WC	No	0
007 WC	No	0
003 PC	No	0
001 PC	No	0
005 PC	No	0
006 PC	No	0

• WC- Well controlled group; PC- Poorly controlled group

⌖VAS-Visual Analogue scores

Table 5. Implant related outcomes at the 3 months post implant placement appointment.

Patient ID and study group •	**Adverse reaction/ Change in medical history**	**ISQ values (Triplicate average)**	**Implant mobility**	**3 months HbA1c %**
004 WC	Instructed to stop DM medication	Mesial 84 Buccal 84	No	11.0 %
002 WC	No	Mesial 87 Buccal 88	No	6.4 %
007 WC	No	Mesial 85 Buccal 85	No	7.6 %
003 PC	No	Mesial 85 Buccal 85	No	7.7 %
001 PC	No	Mesial 85 Buccal 85	No	9.0 %
005 PC	No	Mesial 85 Buccal 85	No	8.6 %
006 PC	No	Mesial 87 Buccal 86	No	8.8 %

- WC- Well controlled group; PC- Poorly controlled group

Table 6. Adverse reactions, change in medical history, peri-implant probing depths and implant mobility at the three months post crown delivery appointment

Patient ID and study group•	**Adverse reaction/Change in medical history**	**Peri-implant probing depth in mm (MB, B, DB, ML, L, DL) **◆**	**Implant mobility (Yes/No)**
004 WC	Placed on metformin again	4(+),3,4,4,2,3	No
002 WC	No	1,3,1,2,3,2	No
003 PC	No	1,2,3,2,3,3	No
007 WC	N/A	N/A	N/A
001 PC	No	2,1,2,1,1,1	No
005 PC	No	2,2,3,3,2,2	No
006 PC	N/A	N/A	N/A
007 WC	N/A	N/A	N/A

• WC- Well controlled group; PC- Poorly controlled group

** Probing depth location on the implant: MB- Mesiobuccal, B- Buccal, DB- Distobuccal, ML- Mesiolingual, L- Lingual, DL- Distolingual

\+ Represents areas with positive bleeding on probing

◆ N/A – Data not available

Table 7. ISQ intergroup comparison between the well controlled and the poorly controlled diabetic groups

Factor **ISQ or Insertion** **torque**	**Well-Controlled group** **(5.8%<HbA1c≥7%)** **n=3**	**Poorly-Controlled group** **(7.5%<HbA1c>10%)** **n=4**	**P-value***
Baseline ISQ **Buccal, mean (SD)**	84.666 (0.577)	82.000 (2.440)	0.13
3 Months ISQ **Buccal, Mean (SD)**	85.666 (2.081)	85.250 (0.500)	0.71
ISQ Buccal change, **mean (SD)**	1.000 (1.732)	3.250 (2.872)	0.29
Baseline ISQ **Mesial, mean (SD)**	85.000 (1.000)	83.250 (2.753)	0.35
3 Months ISQ **mesial, mean (SD)**	85.333 (1.527)	85.500 (1.000)	0.87
ISQ Mesial change, **mean (SD)**	0.333 (0.577)	2.250 (2.217)	0.21
Insertion Torque **N.cm, mean (SD)**	56.666 (5.773)	51.250 (6.291)	0.30

*ISQ values and insertion torque comparison between the well controlled diabetic group and poorly controlled diabetic group were calculated using two sample T-test, $p \leq 0.05$ was considered statistically significant.

Table 8. Hematoxylin & Eosin staining analysis of total bone surface area in mm²

Sample ID And study group•	Area of bone (mm²)				Whole bone surface area (mm²)
	Bottom cancellous 25%	lower middle 25%	upper middle 25%	Top cortical 25%	
004 WC	0.00	0.90	2.65	2.77	7.33
002 WC	2.55	1.95	3.12	2.59	10.21
003 PC	0.00	0.00	3.18	3.33	6.51
001 PC	0.80	1.99	1.39	2.29	6.47
005 PC	0.00	0.00	0.00	3.58	3.58
006 PC	2.68	3.32	3.54	2.78	12.32

• **PC**- Poorly Controlled group (HbA1c 7.5-10.0%); **WC**- Well controlled group 5.8-7.0%

Table 9. Hematoxylin & Eosin staining analysis of adjusted total bone marrow surface in mm²

Sample ID And study group•	Area of bone marrow (mm²)				Whole bone marrow surface area (mm²)
	cancellous 25%	lower middle 25%	upper middle 25%	cortical 25%	
004 WC	0.00	0.03	0.18	0.57	0.73
002 WC	0.07	0.20	0.41	0.08	0.67
003 PC	0.00	0.00	0.66	0.06	0.58
001 PC	0.45	1.51	1.66	0.96	3.57
005 PC	0.00	0.00	0.00	0.11	0.10
006 PC	0.00	0.00	0.00	0.00	0.00

- **PC**- Poorly Controlled group (HbA1c 7.5-10.0%); **WC**- Well controlled group 5.8-7.0%

Table 10. Factor VIII histomorphometrical analysis of adjusted total live tissue area in mm².

Sample ID And study group•	Area of live tissue (mm²)				Whole live tissue area (mm²)
	Cancellous 25%	Lower middle 25%	Upper middle 25%	Cortical 25%	
004 WC	0.00	1.87	5.6	5.75	12.13
002 WC	2.91	3.07	3.26	2.81	10.2
003 PC	0.00	0.00	4.01	4.35	6.67
001 PC	1.97	3.71	3.23	3.18	9.45
005 PC	0.00	0.00	0.00	4.79	4.2
006 PC	2.70	2.94	3.87	3.83	9.4

- **PC**- Poorly Controlled group (HbA1c 7.5-10.0%); **WC**- Well controlled group 5.8-7.0%

Table 11. Factor VIII histomorphometrical analysis of adjusted total blood vessel surface area in mm².

Sample ID And study group•	Area of blood vessels (mm²)				Whole area of blood vessels (mm²)
	cancellous 25%	lower middle 25%	upper middle 25%	cortical 25%	
004 WC	0.000	0.177	0.275	0.179	0.582
002 WC	0.024	0.026	0.051	0.055	0.137
003 PC	0.000	0.000	0.165	0.125	0.240
001 PC	0.001	0.022	0.042	0.050	0.097
005 PC	0.000	0.000	0.000	0.125	0.124
006 PC	0.062	0.024	0.012	0.004	0.074

- **PC**- Poorly Controlled group (HbA1c 7.5-10.0%); **WC**- Well controlled group 5.8-7.0%

Table 12. Number of blood vessels per sample and percent of blood vessel total surface area manually calculated from Factor VIII immunohistochemistry stained samples.

Sample ID And study group•	Number of Blood Vessels per sample	Whole area of blood vessels mm²	Adjusted live tissue area in mm²	Adjusted percent of blood vessel surface area
004 WC	151	0.632	12.13	4.79 %
002 WC	32	0.157	10.2	1.34 %
003 PC	54	0.290	6.67	3.50 %
001 PC	56	0.117	9.45	1.02 %
005 PC	47	0.125	4.2	2.95 %
006 PC	35	0.104	9.4	0.78 %

- **PC**- Poorly Controlled group (HbA1c 7.5-10.0%); **WC**- Well controlled group 5.8-7.0%

Table 13. Adjusted number of blood vessels per mm² for each sample.

Sample ID And study group•	Number of Blood Vessels per mm²
004 WC	11.4
002 WC	2.6
003 PC	6.4
001 PC	4.6
005 PC	9.7
006 PC	2.6

- **PC**- Poorly Controlled group (HbA1c 7.5-10.0%); **WC**- Well controlled group 5.8-7.0%

Table 14. Histological differences between bone core samples taken from the well controlled diabetic group and the poorly controlled diabetic group

Parameters	Well controlled diabetes group (n=2)	Poorly controlled diabetes group (n=4)	P-value
Adipose Tissue/Sample mm², mean (SD)	0.780 (0.014)	1.350 (2.169)	0.74
Bone Surface Area/ Sample mm², mean (SD)	8.770 (2.036)	7.220 (3.666)	0.62
Blood vessels surface area/ Sample mm², mean (SD)	0.395 (0.335)	0.159 (0.088)	0.21
Mean Number of Blood Vessels per mm² (SD)	11.867 mm² (12.350)	8.229 mm² (4.212)	0.58

Comparisons between adipose tissue/sample mm², bone surface area/sample mm², area of blood vessel/sample mm² and mean number of blood vessels per mm² of bone core samples from well controlled diabetic patients and poorly controlled diabetic patients were calculated using two sample t-test, $p \leq 0.05$ was considered statistically significant.

Table 15. Bone core histology analysis adjustment

Sample ID and study group	Whole tissue area in mm^2	Bone dust surface area in mm^2	Percentage of bone dust from samples
004 WC	14.42	1.2	8.3
002 WC	14.23	2.19	15.3
003 PC	10.49	2.13	20.3
001 PC	15.49	3.4	21.9
005 PC	5.47	0.68	12.4
006 PC	19.07	5.73	30