HISTOLOGIC HEALING FOLLOWING TOOTH EXTRACTION WITH SOCKET GRAFTING USING DEMINERALISED FREEZE-DRIED BONE ALLOGRAFT (DFDBA), COMPARED TO UNDISTURBED NORMAL HEALING IN HUMANS: A RANDOMISED CONTROLLED CLINICAL TRIAL

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MASTERS DISSERTATION FOR MScDent

Submitted in fulfilment of the requirements for the degree of MSc in the Department of Periodontics and Oral Medicine , School of Dentistry, Faculty of Health Sciences, University of Pretoria

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Most importantly I thank our Good Lord for granting us the mental and physical abilities to conduct this study in an effort to improve the quality of life of our patients.

DECLARATION

I declare that the research topic ”Histologic healing following tooth extraction with socket grafting using demineralised freeze-dried bone allograft (DFDBA), compared to undisturbed normal healing in humans: a randomised controlled clinical trial” is my own work and that all the sources I have used or quoted have been indicated and acknowledged by means of complete references.

I declare that this work has never been submitted before for any other degree at any other institution.

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ABSTRACT

Aim: With dental implant treatment having evolved into a very regularly applied treatment modality, post-extraction grafting of extraction sockets with DFDBA in an effort to anticipate and pre-empt post-extraction bone loss has become common practice – clinically known as ridge preservation procedures. The aim of this study was however to histologically determine the quality of bone available for implant placement using DFDBA as grafting material in combination with a resorbable collagen membrane, compared to bone in extraction sockets that were left to heal naturally.

Method: Twenty sites were identified from eight patients requiring replacement of two or more extracted teeth by means of dental implant supported structures, on contralateral sides of the same jaw. They received DFDBA grafting of the socket on one side and no grafting on the contralateral side at the time of extraction. When implants were placed 16 – 20 weeks later, core samples of bone from these sites were first harvested by means of a trephine drill and those samples were processed and examined histologically to determine which of these sites displayed better quality of bone.

Results: One patient's samples could not be utilised. Comparing the samples of the remaining nine non-grafted to nine grafted extraction sites, the difference in the calculated percentages of trabecular bone and collagen as well as the numbers of osteocytes, inflammatory cells and blood vessels were statistically insignificant.

Conclusion: The results of the study indicate that statistically there are no significant histological differences between DFDBA-grafted and non-grafted sockets.

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ABBREVIATIONS

UPOHC	University of Pretoria Oral Health Centre
DFDBA	Demineralised Freeze-Dried Bone Allograft
BC	Blood Clot
GT	Granulation Tissue
PM	Provisional Matrix
WB	Woven Bone
LM	Lamellar Bone
TB	Trabecular Bone
DBM	Demineralised Bone Matrix
BMP	Bone Morphogenetic Protein
RG	Remaining Graft

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KEYWORDS

University of Pretoria, Faculty of Health Sciences, School of Dentistry:

University of Pretoria, Faculty of Health Sciences, School of Dentistry is a higher education institution dedicated for the training of dentists, oral hygienists and dental specialists.

Dental Implant Treatment:

Dental implants are a safe, well-established treatment modality implemented to replace missing teeth and support one or more prosthetic teeth. Implants are made of pure titanium or titanium alloy manufactured in a specific way and used to replace roots of teeth – after or at the time teeth are lost. Dental implants are inserted into the jawbone during a surgical procedure.

Bone Grafting:

Bone grafting is a technique used to augment and regenerate lost jawbone. Bone grafts can either be in block form or particulate form and are obtained from different types of donors.

Chapter 1

Introduction

After tooth extraction the dental socket will decrease in volume and change morphologically [1,2]. These changes are clinically significant and can complicate placement of a dental implant. With dental implant treatment becoming so widespread, the need to preserve bone after tooth extraction has become an ever-increasing concern for clinicians [3]. If bone resorption is significant enough, placement of an implant may become extremely challenging, if not impossible. Fortunately, recent advances in bone grafting materials and techniques allow the dentist to place implants in sites that were considered compromised in the past. It is well documented that post-extraction maintenance of the alveolar ridge volume by grafting the socket may minimize ridge resorption and allow placement of an implant that satisfies aesthetic and functional criteria [4,5].

Bone grafting is possible because bone tissue, unlike most other tissues, has the ability to regenerate completely if provided the space into which to grow – with the grafting material ideally enhancing the natural process of osteogenesis. As host bone grows, it will generally replace graft material completely, assisted by new bone growth from vital osteogenic cells within the graft material - resulting in a fully integrated region of new bone [6].

This happens through the process of osteogenesis – which is supported by two distinct processes, namely osteoconduction and osteoinduction. Osteoconduction occurs when bone graft material serves as a scaffold for new bone growth that is maintained by the host bone. Osteoblasts from the margin of the grafting site utilise the bone graft material as a framework upon which to spread and generate new bone. Osteoinduction, on the other hand, involves the stimulation of osteoprogenitor cells to differentiate into osteoblasts, leading to new bone formation – described by Marshall R Urist in a study done in 1965 [7]. This process is facilitated through Bone Morphogenetic Protein (BMP), a growth factor bonded to cell surface receptors that stimulates mesenchymal cells to differentiate into osteoblasts [8,9,10,11]. Growth

factor enhanced grafts are produced using recombinant DNA technology [6]. They consist of either human growth factors or morphogens (BMPs in conjunction with a carrier medium, such as collagen).

Different types of grafting material exist namely autograft, allograft, xenograft and alloplastic.

Autograft comprises of autogenous tissue transplanted from one part to another part in the same individual. Autografts possess osteoconductive, osteoinductive and osteogenic properties – as long as it includes bone marrow and sufficient blood supply in the transplant site [6]. Because it fulfils these three basic requirements of bone regeneration: osteoconduction, osteoinduction and osteogenesis, autogenous bone graft is considered the gold standard in bone regenerative procedures. However, despite these three essential properties, limitations involving autogenous bone grafting - such as the need for second surgery for harvesting of donor bone, significant donor site morbidity, limitations in quantity of bone and the potential for complications, have led to the search and study of alternative materials [10].

Allograft refers to tissue graft that originates from genetically different donors of the same species (compared to the recipient), of which Demineralised Freeze-Dried Bone Allograft (DFDBA) is a common example [6]. DFDBA undergoes sterilisation and deactivation of proteins normally found in healthy bone and is commercially available in different formulations such as blocks, matchsticks, conical shapes and particulate form - which is commonly known as “Bone Sugar” [3]. It involves a process of demineralisation with an agent such as hydrochloric acid, whereby calcium and phosphates are removed, but the osteoinductive extracellular matrix is left - which consists mainly of non-structural proteins, including growth factors such as BMPs and type 1 collagen. Apart from its osteoconductivity, allograft may therefore also have some osteoinductive properties, although these osteoinductive properties may vary significantly between products from different bone banks due to different manufacturing processes [12,13].

Xenograft refers to graft material, chemically processed in a specific way, from a donor of a different species as the recipient, such as bovine, porcine or equine. Xenograft is osteoconductive, but lacks osteoinductive and osteogenic properties [3,6,14].

Alloplastic graft is synthetic graft material, such as hydroxyapatite or tricalcium phosphate [2,15]. Alloplastic grafts are also osteoconductive but without osteoinductive or osteogenic properties.

Bone graft material that has both osteoconductive and osteoinductive properties such as DFDBA may therefore serve as both a scaffold for currently existing osteoblasts and initiate the formation of new osteoblasts, theoretically promoting faster integration of the graft. These supposed properties and the fact that DFDBA is very reasonably priced and easily obtainable makes it an attractive method of bone grafting during implant placement [16].

However, because grafting may introduce added risks of post-operative complications and greater cost to the patient, while benefits are not ensured, it is necessary to determine if DFDBA adds value to the bone healing processes related to implant placement.

This histologic study therefore focuses on the healing patterns of dental extraction sockets after 16 to 20 weeks of healing, with and without the use of commercially available DFDBA. Studies showing that DFDBA results in greater vital bone gain (28% to 53%) compared to mineralised grafting materials (FDBA) (17% to 27%) after three to six months, supports its choice as grafting material [17]. The goal of this study is to histologically compare post-extraction sites that are left undisturbed (control) with those that are grafted with DFDBA (experimental), so that its usefulness in improving bone quality for implant placement can be determined.

Consenting patients needing extraction and implant placement of two or more teeth on opposing sides in the same jaw, received DFDBA grafting of the extraction socket on one side and no grafting of the socket on the other side at time of extractions. When the implants were placed 16 – 20 weeks later, core samples of bone were first harvested by means of a trephine drill, as the first step in the drilling sequence. The harvested samples were then processed and examined histologically to compare the quality of bone.

Chapter 2

Literature review

2.1 Normal physiological socket healing without intervention

Histologically, the extraction of a tooth triggers a cascade of healing processes involving both hard tissue and soft tissue – the hard tissue being alveolar bone and the soft tissues being the periodontal ligament and gingiva.

According to existing literature, what happens is the following [4,16,18]:

- Immediately after a tooth has been extracted, the socket fills with blood up to the gingival margins of the wound, where after the blood clot (BC) starts to develop. In direct contact with the BC are fragments of the mutilated periodontal ligament, which contains substantial quantities of mesenchymal cells, blood vessels and fibrous tissue. The BC consists of erythrocytes and leukocytes embedded in a fibrin network. In the centre of the BC, initially - and later also towards the margin of the blood clot, the erythrocytes start to disintegrate due to coagulative necrosis.

  From the margins of the socket the blood clot is gradually replaced by granulation tissue (GT), which is rich in erythrocytes, inflammatory cells and newly formed vascular structures – with the GT tissue almost entirely replacing and remodelling the blood clot within the first week. Deposition of mineralised tissue begins after this first week of tissue remodelling.
- After two to four weeks, GT and provisional matrix (PM) dominate the tissue fill of the socket by making up between 30% and 50% of the total fill, with typical BC structures no longer being present. Erythrocytes scattered between densely packed mesenchymal cells, collagen fibres and vessels can still be observed, but no or only few scattered inflammatory cells. During this process the residual fibres of the periodontal ligament, which are inserted into the bundle bone, accompany the formation of the PM towards the centre of the extraction socket.
- Within six to eight weeks of healing, the bulk of the fibre bundles of the periodontal ligament together with bundle bone, the GT and BC, are replaced

with PM and primary, or immature, woven bone (WB). WB consists of finger-like projections of immature bone embedded in a primary spongiosa harboured in the marginal portion of the socket, facilitating the progressive mineralisation within the socket by the deposition of an osteoid matrix. After six to eight weeks the PM have been shown to occupy roughly 60% and the WB about 35% of tissue samples - they also dominate in the late phase of healing (12 – 24 weeks), while lamellar bone (LB) is less frequently observed and less represented, if present at all. Therefore, the bone organisation and architecture are often considered not to be complete at 24 weeks after tooth extraction [4,16].

- WB is later replaced by mature, secondary LB, i.e. lamellae of mature, mineralized bone harbouring secondary osteons surrounded by marrow spaces rich in vessels, adipocytes (found in connective tissue), mesenchymal cells and inflammatory cells. LB is further classified as two types: trabecular bone (TB) - also called cancellous or spongy bone, and compact bone (CB) - also called dense or cortical bone. Mature TB is identified by the presence of generally well-defined lamellar regions with lacunae containing osteocyte nuclei. Osteocytes are mature osteoblasts that have become trapped within the bone matrix they produced and continue to form bone to some degree. This is important for maintaining the strength and health of the bone matrix [9,19].

2.2 Socket healing with osteoinductive DFDBA

Due to the fact that the presence of a tooth is crucial for the maintenance of the alveolar process [20], the loss of a tooth and this process of normal post-extraction healing is unfortunately accompanied by a rapid process of bone resorption [10,21] – both in horizontal and vertical dimension, with the greatest loss on the facial and buccal aspects, typically occurring within the first 24 weeks (6 months). Surgically, this poses a challenge in terms of optimal implant positioning in order to achieve optimal functional and aesthetic restoration [1,3].

Studies have shown that the resorption of the alveolus may be countered by grafting of freshly extracted sockets, known as ridge preservation procedures [1,3,5,10,22,23]. Various methods have been described; using autograft, allograft, xenograft or

alloplastic grafting materials in conjunction with or without different resorbable or non-resorbable barrier membranes – of which most procedures have shown to maintain alveolar ridge dimensions after extraction, although there is no evidence to support the superiority of one technique over another [10,16,22,23].

With allograft materials reportedly possessing two of the three basic requirements of bone regeneration, namely osteoconduction – but more specifically osteoinduction, together with a documented history of effectiveness and safety in the mouth - and being commercially produced at low cost in convenient, user-friendly packaging, it is widely used as grafting material of choice [8,11,24]. As mentioned before, studies showing that demineralised DFDBA results in greater vital bone gain after three to six months (28% to 53%) compared to mineralised grafting materials (FDBA) (17% to 27%), support the choice of DFDBA as grafting material [17]. In conjunction, Wood and Mealy have also shown that histologically DFDBA displayed far greater values of new bone formation and less residual graft particles compared to FDBA, with a ratio of 81,26% newly formed vital bone and 18,74% residual graft in favour of DFDBA as opposed to FDBA with only 50,63% new bone formation and 49.37% residual graft material – of the total bone area [16].

Histologically, the residual graft particles (RG) are distinguished from vital TB by the presence of generally well-defined lamellar regions containing lacunae devoid of osteocytic nuclei [8,16]. Sometimes the DFDBA particles are not very well defined, making it difficult to determine exactly where the residual graft particle ends and the new adjacent vital bone begins. The lamellar DFDBA graft particles are usually surrounded by new WB which is characterised by the osteocytes in the lacunae [8]. The PM contains blood vessels, possibly some inflammatory cells, connective tissue and regions of amorphous material known as ‘‘bone dust.’’ This bone dust is created when ground bone is forced into the adjacent marrow spaces during trephine harvesting of the bone cores and when the cores themselves are sliced during tissue processing. The bone dust is regarded as part the PM component – which in the case of DFDBA constitutes almost half the total area. It has no effect on the calculation and quantification of new bone and residual graft particles [1,16]. This ratio between vital bone and RG is an important indicator of the vitality of new bone gained.

As regards timing, Beck and Mealy, 2010, showed no significant difference in the proportions of newly formed bone and residual allograft particles between early

healing (14 weeks) and late healing (27 weeks) [25], validating the chosen time frame of 16 to 20 weeks. Apart from the timing factor, it is also important to bear in mind that the dynamics of new bone formation vary considerably between individuals [4,21].

Chapter 3

Hypothesis, Aim and Objective

3.1 Hypothesis

H(1) DFDBA improves quality of bone in sockets after extraction of teeth.

H(0) DFDBA does not improve quality of bone in sockets after extraction of teeth.

3.2 Aim

The aim of this study was to histologically compare the quality of bone achieved after DFDBA grafting of extraction sockets with ungrafted extraction sockets.

3.3 Objective

The objective was to ascertain whether there is any possible bone quality advantage in augmenting dental extraction sockets by utilising a technique of DFDBA grafting in combination with a collagen membrane – as opposed to normal undisturbed healing, in the same jaw from the same patient. The generally accepted parameters indicating new bone formation, namely inflammatory cell count, blood vessel count, collagen estimate, trabecular bone quality, osteocyte count and remaining graft were to be used. Samples of bone from both sockets were analysed histologically and compared to determine which of the sites displayed a better quality of healed bone to possibly ensure greater implant stability and better integration

Chapter 4

Methods

4.1 Study design

The study is designed as a randomised (controlled) clinical trial investigating the histologic difference in bone quality after healing between non-grafted sockets and sockets grafted with DFDBA and a resorbable membrane.

4.2 Setting

The study was conducted by the author as investigator, both in private practice and the School of Dentistry, Faculty of Health Sciences, University of Pretoria.

4.3 Sample selection

Basic criteria for selection were patients requiring at least two non-molar extractions, simultaneously, within the same jaw, with planned subsequent dental implant placement.

To obtain 20 sites, the initial plan was to ideally involve ten subjects with two sites each, but within the constraints of private practice, where the clinical part of the study was performed, adjustments were anticipated.

The following additional criteria were required:

4.3.1 Inclusion Criteria:

Patients had to be at least 18 years old and given voluntary consent to participate in the study.

Single-rooted non-molar teeth due for extraction - with radiological evidence of sufficient bone support and tooth orientation conducive to ideal implant placement, were selected to ensure adequate depth of socket for harvesting of a core biopsy without including surrounding native bone [4].

4.3.2 Exclusion Criteria:

Multirooted teeth were excluded because of the possibility of interradicular bone being harvested as well, as well as sockets with severe dehiscences. This meant that the cores were taken from stable, well healed sockets and all slices utilised for histological analysis were taken from the centres of the cores.

The following were also excluded:

- Patients with an impaired immune system due to autoimmune disease or immunosuppressive treatment.
- Patients with an uncontrolled systemic disease, such as uncontrolled hypertension or uncontrolled diabetes.
- Patients on long-term anti-inflammatory drug therapy.
- Patients with a history of allergy to DFDBA or collagen membranes.
- Teeth with periapical pathology.
- Extensive bone loss during extraction process.

4.4 Clinical Process

- Medical history and demographical information were obtained from the clinic's standard patient questionnaires as well as personal interviews. Qualifying patients were given an introductory letter and consent form stating the purpose of the research (Appendix F together with Appendix G).
- After giving informed consent, a unique study number was assigned to each patient which was linked to the results of the clinical trial.

Table 4.1: DFDBA project – sample identification.

PATIENT	CASE NR	SITE	SITE NR	WITH/-OUT DFDBA
PST 1 (Mx 97527)	001-231-2016	11 13	001-231-2016-11 001-231-2016-13	Natural Healing DFDBA Grafted
PST 2 (Mx 106283)	002-231-2016	33 43	002-231-2016-33 002-231-2016-43	Natural Healing DFDBA Grafted
PST 3 (Mx 14636)	004-231-2016	33 43	004-231-2016-33 004-231-2016-43	Natural Healing DFDBA Grafted
PST 4 (Mx 96928)	005-231-2016	33 43	005-231-2016-33 005-231-2016-43	Natural Healing DFDBA Grafted
PST 5 (Mx 57121)	006-231-2016	33 43	006-231-2016-33 006-231-2016-43	DFDBA Grafted Natural Healing
PST 6 (Mx 95370)	007-231-2016	33 43	007-231-2016-33 007-231-2016-43	DFDBA Grafted Natural Healing
PST 7 (Mx 111758)	008-231-2016	13 21	008-231-2016-13 008-231-2016-21	Natural Healing DFDBA Grafted
PST 8 (Mx 000224)	009-231-2016	13 23	009-231-2016-13 009-231-2016-23	Natural Healing DFDBA Grafted
PST 8 (Mx 000224)	009-231-2016	15 25	009-231-2016-15 009-231-2016-25	Natural Healing DFDBA Grafted
PST 8 (Mx 000224)	009-231-2016	34 44	009-231-2016-34 009-231-2016-44	DFDBA Grafted Natural Healing

- Intra-oral examination, peri-apical and panoramic radiological images and Cone Beam Computerised Tomography (CBCT) scans were performed pre-operatively.
- If deemed necessary, customised acrylic occlusal stents were fabricated on study models to serve as fixed reference guides for both accurate harvesting of core samples and subsequent placement of implants.
- Intra-operatively the relevant teeth were removed utilising a low-trauma technique to ensure preservation of socket walls.
- Two of the subjects needed treatment where fractured roots had to be removed due to failing bridges (Figures 4.1 & 4.2).

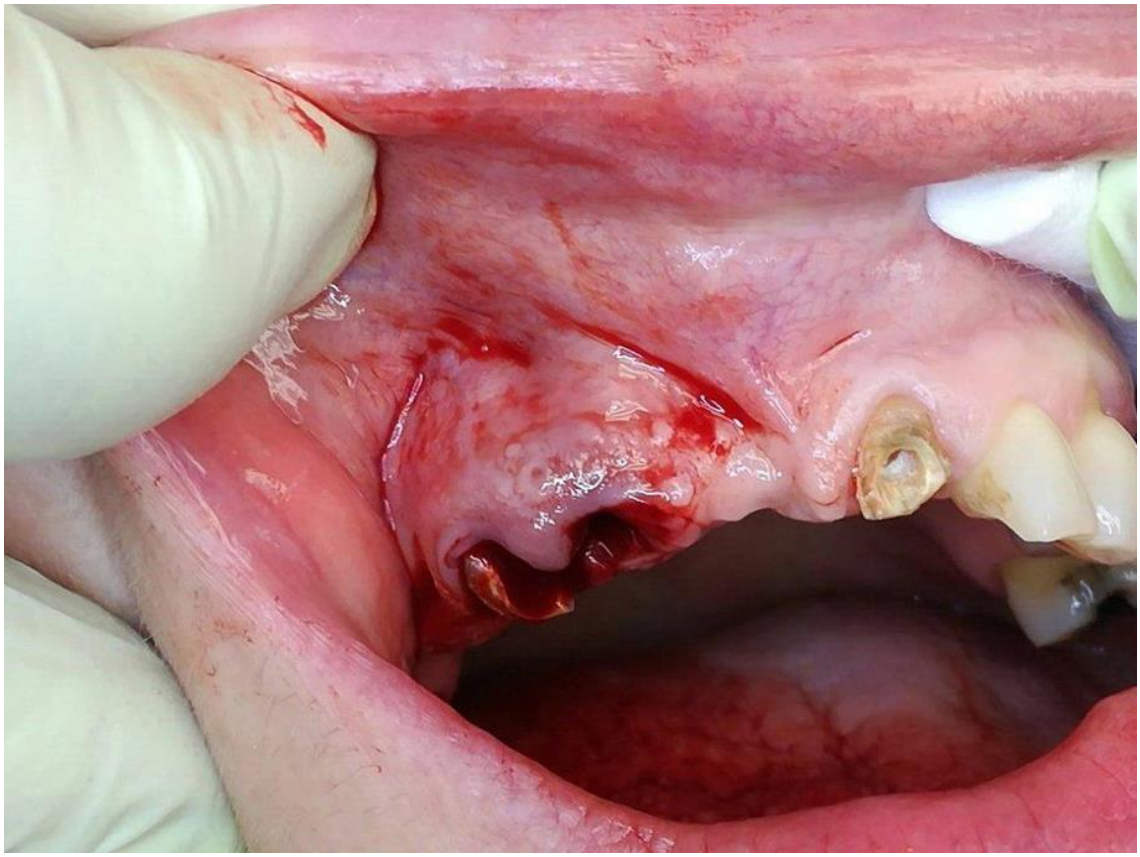

Figure 4.1: Root remnants in situ.

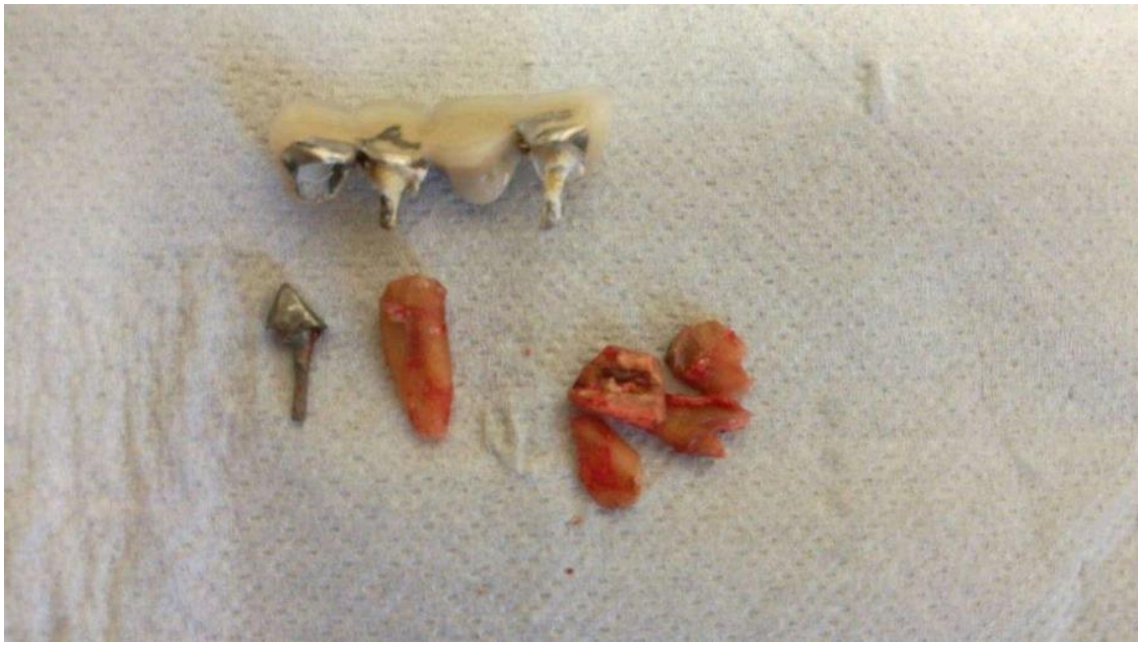

Figure 4.2: Root remnants after having removed failed bridge.

whereas the rest of the subjects were all candidates for dentectomies necessitated by a failing dentition (Figure 4.3).

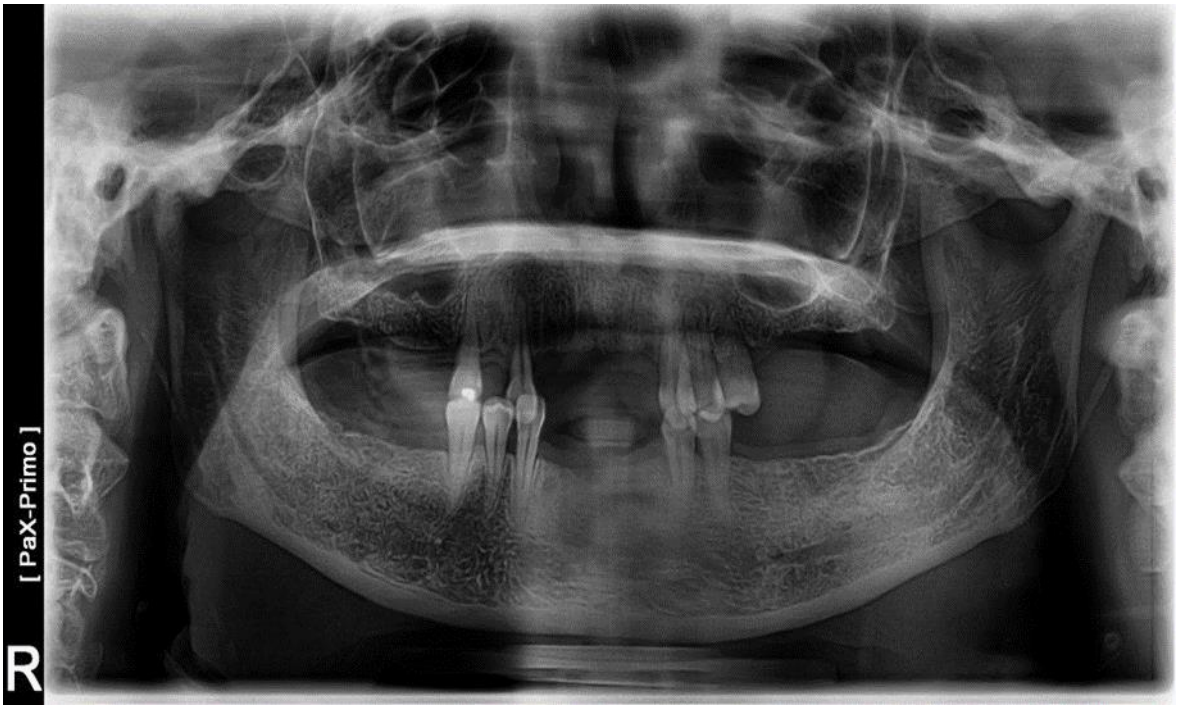

Figure 4.3: Panoramic image of failing dentition.

The random allocation of which sockets to graft with DFDBA and which to leave undisturbed was done by the flip of a coin with the patient as witness, purely because it is and has always been regarded as a simple, seemingly unbiased, method of deciding between 2 options and is being used regularly in scientific studies [20]. In the DFDBA graft group a full-thickness gingival flap was raised to expose both labial and lingual/palatal aspects of the alveolar ridge before commencement of tooth removal.

- After tooth removal and placement of the DFDBA grafting material, a resorbable collagen membrane was placed to completely cover the socket and extend to a minimum of 3mm beyond the alveolar crest - where after the gingival flap was replaced and sutured with monofilament non-resorbable sutures [1]. The membrane acts as a barrier against the ingrowth of soft tissue into the healing site and helps to prevent loss of the grafting material. Current clinical trends tend to favour the use of resorbable membranes, although the study of different types of bone substitution materials combined with different types of membranes is ongoing and their efficacy in obtaining optimal results in immediate extraction socket preservation still need to be defined [20]. The DFDBA was supplied by the National Tissue Bank of the University of Pretoria

(ISO 9001:2000 and ISO 13485:2003) with the collagen membrane being a "Jason Membrane" from Botiss Biomaterials.

- Post-operatively all patients received the same prescription of a 0,2% chlorhexidine rinse twice daily for ten days, the same antibiotic regime of Clindamycin 150mg four times a day for four days and the same analgesics as needed for four days. Clindamycin was chosen due to its effectiveness in both soft tissue and bone infections and also because none of the subjects reported to be allergic to Clindamycin. The analgesic of choice was a standard composition containing 400mg Ibuprofen and 325mg Paracetamol – providing analgesic, anti-inflammatory and antipyretic action.
- Sutures were removed after ten days. All cases displayed excellent and uneventful healing at that stage and by mutual agreement it was decided to do four-weekly follow-ups instead of bi-weekly, as was stipulated in the informed consent, until implant placement 16 - 20 weeks after removal of teeth. The quality of bone was assessed 16 to 20 weeks after grafting because Beck and Mealy, 2010 [5], demonstrated that allografted sites did not yield greater bone formation at 24 weeks as opposed to 12 weeks. However, new bone formation is time and subject dependent [2,4,10], but these variables were eliminated in this study by each patient serving as his own control.
- To ensure that only bone from the extraction socket was harvested and also not to compromise primary stability of the implants, at re-entry core samples of at least 8mm (but no longer than10 mm) in length were harvested by means of a 3,6mm internal diameter trephine, with abundant water supply to prevent overheating of the bone, as the first step in the implant placement drill sequence (Figure 4.4). The cores were removed from the trephine using a thymosin probe placed into the window of the bur to displace the material. These harvested cores were then stored in a 10% neutral buffered formalin solution in numbered containers (Figure 4.5).

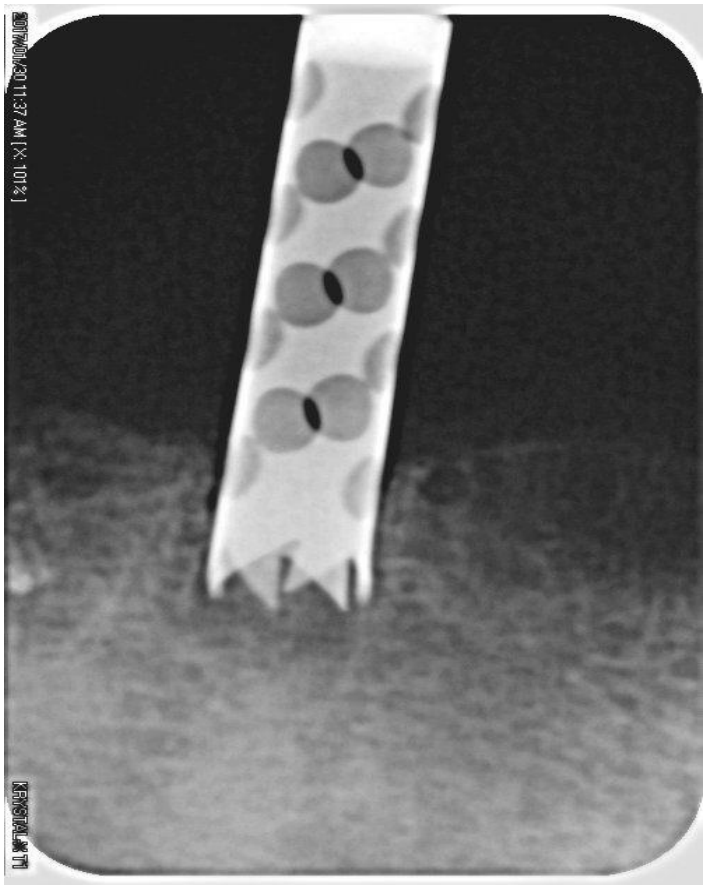

Figure 4.4: X-Ray image of trephine drill.

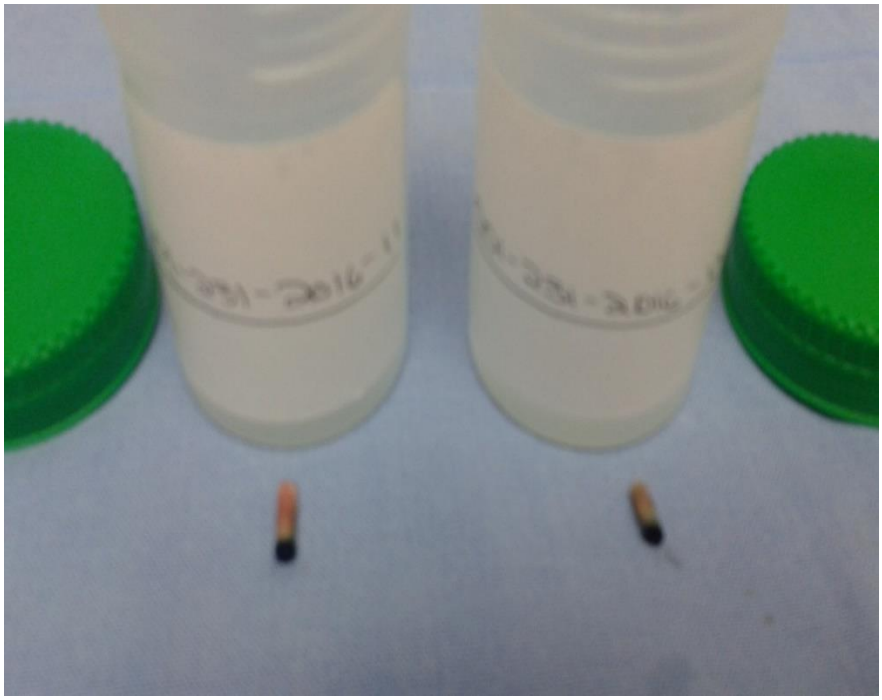

Figure 4.5: Harvested core samples with numbered containers.

After harvesting of the core biopsies, the final osteotomies were prepared and each of the sites received a dental implant (Neodent, Straumann Group) with good primary stability established in each case.

Images of each harvested core specimen were digitally captured and examined to differentiate between the parameters as described before.

4.5 Data collection

4.5.1 The trephine core samples were prepared and processed for histological analysis by the Department of Oral Pathology and Oral Biology, School of Dentistry, Faculty of Health Sciences at the University of Pretoria, by decalcification and embedding in paraffin wax, after which they were sectioned and stained with haematoxylin and eosin dye. Two 5µm thick slices were taken lengthwise coronally to apically from the centre of each trephine core to obtain, under 4x magnification, the percentage collagen, trabecular bone, percentage remaining graft material and number of osteocytes and under 20x magnification the number of blood vessels and inflammatory cells.

4.5.2 The two slices of each of the 20 core samples were digitally photo-documented using a Leica DMD108 (DMD= DigitalMicroimagingDevice) Microscope (Leica, Germany) and the best of the two was then utilised to conduct the rest of the study. Some of the slices tore and folded quite considerably during processing and were therefore discarded. Four images (with a scale bar) of each slice were captured: three under 4x magnification - one from each extremity and one from the centre, covering the whole of the sample and one under 20x magnification from the centre of the core, providing a total of 80 digital images, which were then saved on a memory stick.

As regards the counting of the osteocytes (under 4x magnification) and inflammatory cells (under 20x magnification), various commercially available computerised image analysis software products were considered, but due to the variations in shape and size of the cells the grid method was opted for. Instead of the traditional microscope reticle grid though, a 10x10 grid (100 blocks per grid) was created in Microsoft Word and each digital image was imported into the grid and numbered according to the unique study number allocated to each patient. (Figure 4.6 and Figure 4.7).

Using the scale bar (= 1mm) as reference, the size of one grid block of the 4x magnification slices was calculated to be 0.080mm² (Figure 4.6).

Each block of the 20x magnification slides (scale bar = 100µm) was calculated to be 3 071,75µm² (Figure 4.7).

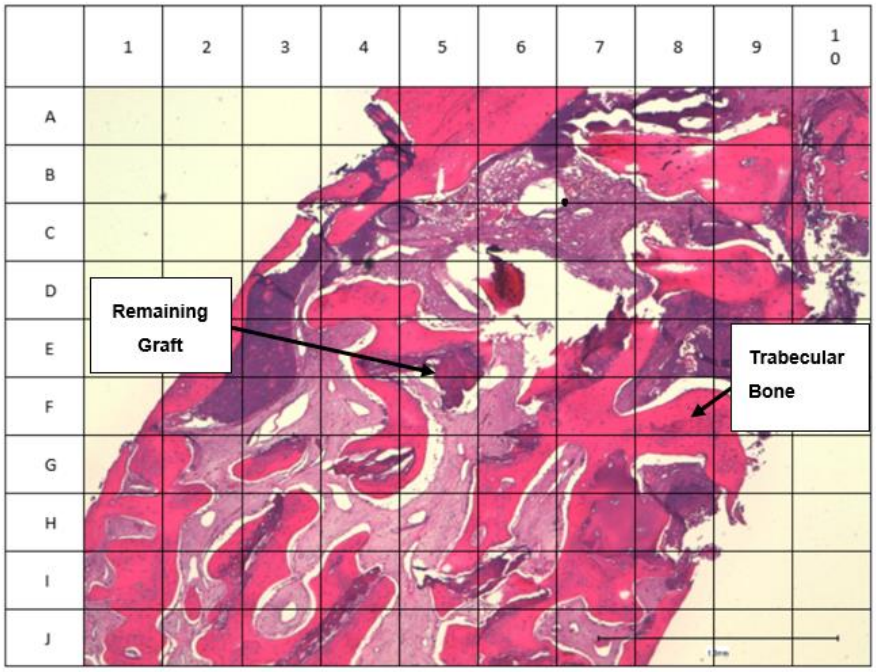

Figure 4.6: Example of the grid with the imported image (4x magnification).

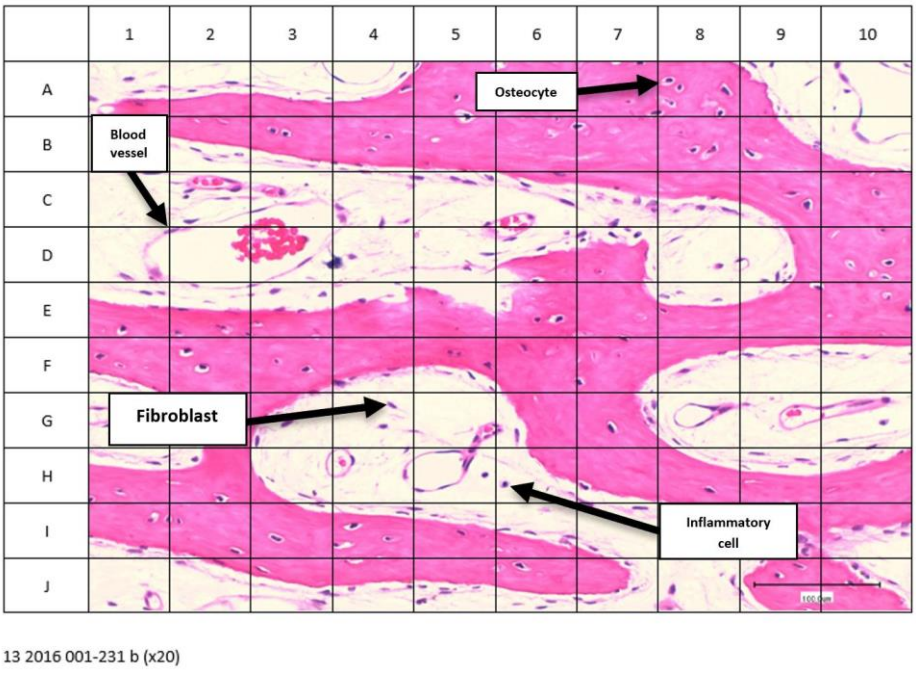

Figure 4.7: Example of the grid with the imported image (20x magnification).

A copy of each slice was printed to check for and eliminate overlaps in order to prevent duplication, resulting in a total of 7200 data containing blocks.

The slices were evaluated for the previously mentioned histological parameters of osteogenesis [15,26,27] by counting the number of osteocytes as well as calculating/estimating percentages of trabecular bone, collagen and RG under 4x magnification and then counting the number of inflammatory cells and blood vessels under 20x magnification.

To ensure meaningful data the 20x grid (as in Figure 4.7) was divided into two identical sections, each consisting of 50 of the smaller blocks – resulting in a block size of 3 071,75 µm² x 50 = 153 587,5µm² (0.1535875mm²). Evaluation was done by viewing the Microsoft Word grid images on a computer screen, magnified to 500%. This large magnification can sometimes cause a loss of resolution or sharpness, so a normal light microscope (Aomekie Student Microscope, China) was used to verify images.

The data obtained was captured in Microsoft Excel spreadsheet (as described below) in the following columns:

- Case Number.
- Site.
- Slide number (typically 4 slides per site, numbered: 1, 2, 3, 4).
- Graft (1 = Yes; 0 = No).
- Grid Block Number (Refer to Figure 4.3 and 4.4; eg, A1, A2, B1, B2, etc).
- Data coverage estimate (2 units = 100%; 1 unit = partial coverage (1-99%); * = no data). This was necessary because grid blocks contained varying amounts of data, ranging from no data to 100% data. This estimate enabled mathematical adjustment of estimates and counts because of sample variances.
- Inflammatory cell count.
- Blood vessel count.
- Collagen category (subjective estimate of the percentage surface area containing collagen:
  0 = no collagen; 1 = ≤33.3%; 2 = >33.3%-66.7%; 3 = >66.7%).
- Collagen estimate (subjective estimate of the percentage surface area containing collagen).

- Trabecular bone category (subjective estimate of the percentage surface area containing trabecular bone:
  0 = no trabecular bone; 1 = ≤33.3%; 2 = >33.3%-66.7%; 3 = >66.7%).
- Trabecular bone estimate (subjective estimate of the percentage surface area containing trabecular bone).
- Osteocyte count.
- Remaining graft (RG) material category (subjective estimate of the percentage surface area containing remaining graft material:
  0 = no remaining graft; 1 = ≤33.3%; 2 = >33.3%-66.7%; 3 = >66.7%).
- Remaining graft (RG) estimate (subjective estimate of the percentage surface area containing remaining graft).

The co-supervisor controlled the integrity of the datasheet and the primary investigator corrected a minority of initial input errors through recounting.

After all the counting was concluded, inflammatory cell, blood vessel and osteocyte counts were summed per site. Counts per grid block were calculated by taking the variable data coverage in the slides into account. Since “2” indicated 100% the data coverage in a block, the total was divided by two to obtain a value from zero to one. This value was in turn used to adjust the counts to reflect a more accurate account of the proportional differences on average per block.

The categorical estimates (0, 1, 2 and 3) for collagen, trabecular bone and remaining graft were totalled, using the “Countif” function in Excel that enabled the calculation of percentage distributions for each category. The percentage distributions were in turn used to calculate a total estimate for each case, using the numerical midpoint of each category as the utility weight. This method will hence forth be referred to as Estimate 1. In addition to this the mean score of the collagen, trabecular bone and remaining graft subjective percentage estimates by the primary investigator were recorded as the second value in this regard. This method will hence forth be referred to as Estimate 2. It was decided to use two different methods to estimate the prevalence of tissue types because of the subjectivity of the measurement and the lack of any existing methods that can perform this measurement objectively. It can be argued that if there

is strong correlation between the two different ways of measurement then it would indicate that there is some reliability in the methods.

4.5.3 Sample identification was done by the author and randomly controlled by the supervisor.

4.5.3.1 Inter-examiner reliability testing: The primary supervisor of this project repeated the counts and estimates of 72 randomly selected grid blocks. The Random function in Microsoft Excel was used to isolate the 72 records.

4.5.3.2 Intra-examiner reliability testing: The primary investigator of this project repeated the counts and estimates of 72 selected grid blocks that was identified using the same methods as described, above.

During this process, a practical problem was encountered under the 20x magnification in that it was often confusing and difficult to distinguish between inflammatory cells and fibroblasts. This became evident due to the conflicting numbers of the author and the random checks of the supervisor. The only option was to re-count; four independent re-counts of all data by the author and two re-counts by the supervisor, where after the closest matching results were utilised.

4.6 Data analysis

The data analysis consisted of descriptive statistics to compare differences in counts between grafted and non-grafted sites and paired t-tests or appropriate non-parametric equivalent analyses (Wilcoxon Sign Rank Test) to establish statistical significance. Significance was set at 0.05.

The inter-class correlation coefficient was used to report the intra- and inter-rater agreement.

4.7 Ethical considerations

The relevant authority, namely the Chair of the School of Dentistry, University of Pretoria, gave consent for the study to be conducted at the University of Pretoria Oral Health Centre (UPOHC) (Appendix A).

A proposal for the project was submitted to and approved by the University of Pretoria, Faculty of Health Sciences Research Ethics Committee on 30 June 2016 – Reference nr 231/2016 (Appendix B) and renewed on 18 June 2019 (Appendix C).

All personal data was kept confidential and patient anonymity was respected. Patient files and data will be stored in the dental practice archives for no less than 15 years until 31 December 2033. Completed data storage forms to be attached to patient files (Appendix H).

Chapter 5

Results

5.1 Sample realisation

Eight patients requiring at least two non-molar extractions in the same jaw and one patient requiring three non-molar extractions in both upper and lower jaws, were finally selected to participate. This sample yielded ten sites for natural healing (control) and ten sites grafted with DFDBA and a collagen membrane (experimental). Control and experimental sites were randomly determined by the flip of a coin. After processing of the core samples, it was found that the samples of one patient (Subject 7) could not be utilised because of hundred percent connective tissue formation with no bone healing. This resulted in a total of nine grafted and nine non-grafted sites. Six subjects had two sites each and one subject had six sites (four maxillary and two mandibular) totalling 18 sites.

The sample comprised one male and seven females with ages ranging between 30 and 68, with a mean age of 54,87 (Table 5.1). The information obtained from subject no 7 was discarded.

Table 5.1: Age and gender distribution of the sample.

CASE / GENDER	AGE (YEARS)
1 – Female	56
2 – Female	30
3 – Female	51
4 – Female	68
5 – Male	68
6 – Female	52
7 – Female	60
8 – Female	54

Upon re-entering of the sites, one of the subjects produced only connective tissue in the coronal 8mm of the non-grafted site and that histological data had therefore to be eliminated from the study. The remaining 18 sites were histologically analysed with nine biopsies in each group. The DFDBA grafted group consisted of two maxillary canines, one maxillary second premolar, one mandibular first premolar and five mandibular canines, whereas the non-grafted group consisted of one maxillary central incisor, one maxillary canine, one maxillary second premolar, one mandibular first premolar and five mandibular canines. The majority of the sites (twelve) were from the mandible and the balance (six) from the maxilla.

Clinically, there was no loss of graft material at the four-week follow-up appointments and all the sites were healing without complication.

5.2 Collagen Estimates

Table 5.2: Pairwise comparison of collagen estimates for sites where a graft was placed, or not.

PATIENT	SITE ID(TOOTH NUMBER)	GRAFT PLACED	COLLAGEN %ESTIMATE 1(DIFFERENCE)	COLLAGEN %ESTIMATE 2(DIFFERENCE)
1	11	No	31	29
1	13	Yes	28 (-3%)	26 (-3%)
2	33	No	9	8
2	43	Yes	21 (+12%)	20 (+12%)
3	33	No	49	48
3	43	Yes	47 (-2%)	47 (-1%)
4	33	No	37	34
4	43	Yes	32 (-5%)	29 (-5%)
5	43	No	43	29
5	33	Yes	37 (-6%)	35 (+6%)
6	43	No	29	25
6	33	Yes	28 (-1%)	25 (0%)
7	13	No	72	82
7	21	Yes	36 (-36%)	35 (-47%)
8	13	No	28	24
8	23	Yes	22 (-6%)	20 (-4%)
8	15	No	33	32
8	25	Yes	36 (+3%)	35 (+3%)
8	44	No	23	22
8	34	Yes	27 (+4%)	25 (+3%)

Note Patient 7 excluded from pairwise comparison
Collagen Estimate 1: Wilcoxon Sign Rank Test: P=0.0594
Collagen Estimate 2: Wilcoxon Sign Rank Test: P=0.594
Pearson Correlation Coefficient for Collagen Estimate 1 and Collagen Estimate 2: (r): 0.967
Median of Collagen Estimate 1 =31%
Median of Collagen Estimate 12 =29%
ICC (Inter-rater agreement): 0.98 (95%CI:0.97-0.99; P=0.000)
ICC (Intra-rater agreement): 0.99 (95%CI:0.99-1.00; P=0.000)

An erratic pattern emerged with no conclusive link between estimated collagen percentages for grafted and non-grafted sites (Table 5.2, Wilcoxon Sign Rank Test: P=0.0594).

5.3 Trabecular Bone Estimates

Table 5.3: Pairwise comparison of trabecular bone estimates for sites where a graft was placed, or not.

PATIENT	SITE ID (TOOTH NUMBER)	GRAFT PLACED	TRABECULAR BONE % ESTIMATE 1 (DIFFERENCE)	TRABECULAR BONE % ESTIMATE 2 (DIFFERENCE)
1	11	No	47	45
1	13	Yes	36 (-11%)	36 (-9%)
2	33	No	55	55
2	43	Yes	40 (-15%)	38 (-17%)
3	33	No	39	36
3	43	Yes	27 (-12%)	26 (-10%)
4	33	No	42	40
4	43	Yes	37 (-5%)	35 (-5%)
5	43	No	36	35
5	33	Yes	36 (0%)	34 (-1%)
6	43	No	34	32
6	33	Yes	35 (+1%)	33 (+1%)
7	13	No	1	1
7	21	Yes	36 (+35%)	34 (+33%)
8	13	No	38	36
8	23	Yes	44 (+6%)	43 (+7%)
8	15	No	41	38
8	25	Yes	35 (-6%)	33 (-5%)
8	44	No	48	46
8	34	Yes	38 (-10%)	35 (-11%)

Note Patient 7 excluded from pairwise comparison
Trabecular Bone Estimate 1: Wilcoxon Sign Rank Test: P=0.051
Trabecular Bone Estimate 2: Wilcoxon Sign Rank Test: P=0.051
Pearson Correlation Coefficient for Trabecular Bone calculated and Trabecular Bone Estimate (r): 0.997
Median of calculated Trabecular Bone Estimate 1=37%
Median of estimated Trabecular Bone Estimate 2=35%
ICC (Inter-rater agreement): 0.94 (95%CI:0.91-0.96; P=0.000)
ICC (Intra-rater agreement): 0.94 (95%CI:0.90-0.96; P=0.000)

Table 5.3 illustrates that six of the sites displayed between 5% and 15% less trabecular bone in the grafted sockets, two of the sites displayed 1% and 6% more trabecular bone in the grafted sockets and one site displayed zero difference. These results were however not statistically significant (Wilcoxon Sign Rank Test: P=0.051).

5.4 Osteocyte Counts

Table 5.4: Pairwise comparison of osteocytes counted for sites where a graft was placed or not.

PATIENT	SITE ID (TOOTH NUMBER)	GRAFT PLACED	ADJUSTED NUMBER OF DATA UNITS	TOTAL OSTEOCYTES COUNTED	OSTEOCYTES PER 0,08MM² BLOCK	OSTEOCYTES /MM²	DIFFERENCE
1	11	No	176.0	2381	13.53	169.11	
1	13	Yes	103.0	1528	14.83	185.44	16.33
2	33	No	106.5	3477	32.65	408.1	
2	43	Yes	101.5	1862	18.34	229.31	-178.79
3	33	No	194.0	4236	21.84	272.94	
3	43	Yes	101.5	1392	13.71	171.43	-101.51
4	33	No	116.0	3060	26.38	329.74	
4	43	Yes	106.0	2185	20.61	257.67	-72.07
5	43	No	184.5	3253	17.63	220.39	
5	33	Yes	139.0	2607	18.76	234.44	14.05
6	43	No	73.0	1458	19.97	249.66	
6	33	Yes	81.0	1209	14.93	186.57	-63.09
7	13	No	75.5	15	0.20	2.48	
7	21	Yes	164.0	2756	16.80	210.06	207.58
8	13	No	109.0	1574	14.44	180.5	
8	23	Yes	109.0	2451	22.49	271.08	90.58
8	15	No	98.5	2094	21.26	265.74	
8	25	Yes	128.0	2606	20.36	254.49	-11.25
8	44	No	143.5	3878	27.02	337.8	
8	34	Yes	106.5	3204	30.08	376.06	38.26

Note Patient 7 excluded from pairwise comparison

Wilcoxon Sign Rank Test: P=0.441

Median of osteocytes counted/block=19.364

ICC (Inter-rater agreement): 0.97 (95%CI:0.95-0.98; P=0.000)

ICC (Intra-rater agreement): 1.00 (95%CI:0.99-1.00; P=0.000)

Referring to Table 5.4, varying patterns of osteocyte prevalence were observed without any direct gradient leaning towards grafted or non-grafted sites (Wilcoxon Sign Rank Test: P=0.441).

5.5 Remaining Graft Material Estimates

Table 5.5: Descriptive display of graft remnant %.

PATIENT	SITE ID (TOOTH NUMBER)	GRAFT PLACED	% REMAINING GRAFT REMNANTS ESTIMATE 2*
1	13	Yes	2
2	43	Yes	1
3	43	Yes	4
4	43	Yes	1
5	33	Yes	3
6	33	Yes	1
7	21	Yes	4
8	23	Yes	2
8	25	Yes	1
8	34	Yes	0

Table 5.5 shows that between 1% and 4% of graft material remained.

5.6 Inflammatory Cell Counts

Table 5.6: Pairwise comparison of inflammatory cells counted for sites where a graft was placed or not.

Patient	Site ID (tooth number)	Graft placed	Adjusted number of Data units	Inflammatory cells counted	Inflammatory cells per 0.154mm² block	Inflammatory cells /mm² (Difference)
1	11	No	176.0	18	0.10	0.67
1	13	Yes	103.0	33	0.32	2.09 (+1.42)
2	33	No	106.5	0	0.00	0.00
2	43	Yes	101.5	20	0.20	1.28 (+1.28)
3	33	No	194.0	8	0.04	0.27
3	43	Yes	101.5	20	0.20	1.28 (+1.01)
4	33	No	116.0	26	0.22	1.46
4	43	Yes	106.0	3	0.03	0.18 (-1.28)
5	43	No	184.5	7	0.04	0.25
5	33	Yes	139.0	9	0.06	0.42 (+0.17)
6	43	No	73.0	18	0.25	1.61
6	33	Yes	81.0	67	0.83	5.39 (+3.78)
7	13	No	75.5	0	0.00	0.00
7	21	Yes	164.0	0	0.00	0.00
8	13	No	109.0	2	0.02	0.12
8	23	Yes	109.0	12	0.11	0.72 (+0.60)
8	15	No	98.5	6	0.06	0.40
8	25	Yes	128.0	29	0.23	1.48 (+1.08)
8	44	No	143.5	11	0.08	0.50
8	34	Yes	106.5	23	0.22	1.41 (+0.91)

Note Patient 7 excluded from pairwise comparison
Related Samples Wilcoxon Sign Rank Test: P=0.051
ICC (Inter-rater agreement): 0.81 (95%CI:0.50-0.93; P=0.000)
ICC (Intra-rater agreement): 0.76 (95%CI:0.49-0.96; P=0.001)

Referring to Table 5.6, it can be seen that in most instances there were more inflammatory cells present when a graft was placed. These differences were however not statistically significant (Wilcoxon Sign Rank Test: P=0.051).

5.7 Blood vessels

Table 5.7: Pairwise comparison of blood vessels counted for sites where a graft was placed or not.

PATIENT	SITE ID (TOOTH NUMBER)	GRAFT PLACED	ADJUSTED NUMBER OF DATA UNITS	BLOOD VESSELS COUNTED	BLVES PER 0.1535875MM² BLOCK	BLOOD VESSELS /MM² (DIFFERENCE)
1	11	No	176.0	2	0.01	0.07
1	13	Yes	103.0	8	0.08	0.51 (+0.44)
2	33	No	106.5	0	0.00	0.00
2	43	Yes	101.5	5	0.05	0.32 (+0.32)
3	33	No	194.0	12	0.06	0.40
3	43	Yes	101.5	5	0.05	0.32 (-0.08)
4	33	No	116.0	7	0.06	0.39
4	43	Yes	106.0	4	0.04	0.25 (-0.14)
5	43	No	184.5	9	0.05	0.32
5	33	Yes	139.0	14	0.10	0.66 (+0.34)
6	43	No	73.0	6	0.08	0.54
6	33	Yes	81.0	5	0.06	0.42 (-0.12)
7	13	No	75.5	0	0.00	0.00
7	21	Yes	164.0	0	0.00	0.00
8	13	No	109.0	1	0.01	0.06
8	23	Yes	109.0	8	0.07	0.48 (+0.42)
8	15	No	98.5	6	0.06	0.40
8	25	Yes	128.0	13	0.10	0.66 (+0.26)
8	44	No	143.5	6	0.04	0.27
8	34	Yes	106.5	3	0.03	0.18 (-0.09)

Note Patient 7 excluded from pairwise comparison
Related Samples Wilcoxon Sign Rank Test: P=0.139
Median of blood vessels counted/block (no graft vs graft)=0.049 vs 0.062
ICC (Inter-rater agreement): 0.86 (95%CI:062-0.95; P=0.001)
ICC (Intra-rater agreement): 0.76 (95%CI:0.37-0.94; P=0.001)

Referring to Table 5.7, no consistent gradient could be observed for blood vessel counts between grafted sites and non-grafted sites (Wilcoxon Sign Rank Test: P=0.139).

The overall results therefore indicate that there were no significant histological differences for any of the parameters tested between the grafted and non-grafted groups.

Chapter 6

Discussion

Dental implant treatment aims to restore form and function of the dentally compromised patient by providing support to prosthetic over-structures. Sufficient volume and quality of bone is necessary for anchoring the implant. While the goal of DFDBA placement in extraction sockets is to preserve the volume of bone available for implant placement [1, 2, 5, 6, 10, 12 13, 15], it is important to determine the quality of bone achieved through this grafting procedure [1,13]. Based on this premise, this study therefore aimed to histologically compare dental extraction sites grafted with DFDBA with non-grafted sites before implant placement. The comparison was done by assessing the following parameters of osteogenesis: number of osteocytes, percentages of trabecular bone, collagen and remaining graft material, the number of inflammatory cells and blood vessels [7,22,23].

It is pertinent to note that this study could not show a meaningful statistical difference for the six histological parameters of osteogenesis between grafted and non-grafted sockets. It stands in contrast to the reportedly osteoinductive properties of DFDBA, which could possibly be ascribed to the specific product that was used, although it was sourced from a very reputable supplier. A study by Schwarz et al in 1996 [13] showed that there could be major differences in DFDBA preparations produced by different commercial bone banks and their ability to induce new bone, due to the use of various bone manufacturing methods. Factors such as particle shape and size, the pH of the solution and varying types and levels of BMPs have been studied and shown to have an influence on the degree of osteoinductivity of different DFDBA products [6,18,24,25].

Table 5.6 showed more inflammatory cells in grafted areas compared to non-grafted areas. Although these differences were not statistically significant (Wilcoxon Sign Rank Test: P=0.051), such gradients are not surprising and can be interpreted as an indicator of the response of the human body to the introduction of foreign material. Higher sample size may have rendered statistically significant results.

Moreover, in Table 5.5, it was shown that between 1% and 4% of graft material remained after 16 to 20 weeks, indicating that basically virtually all of the DFDBA have been replaced by trabecular bone, which correlates time frames suggested by Beck and Mealy, 2010 [25]. The outcome of this study therefore suggests that at 16 to 20 weeks after extraction, most graft material have been replaced by bone but no additional benefit in terms of bone quality could be confirmed. This finding is consistent with the findings of a randomised control trail reported by Brownfield and Weltman in 2012 [12].

The findings of this study should be interpreted with caution. Major limitations of this study include the small sample size, that can be attributed to the logistical constraints of private practice, and extensive amount of time required to quantify the parameters. The seven subjects who finished the study were however regarded as a good cross-section of the average patient attending a dental practice requiring restoration of either function or aesthetics or both – to a lesser or greater degree.

It should be noted that the study also did not intentionally differentiate between males and females or upper and lower jaws. The subjects' age was also not taken into account. Similar to other studies this study also did not distinguish between smokers and non-smokers. Although these omissions can be considered as limitations it was deemed not necessary. The idea was to compare grafted to non-grafted sockets within the same individual so that the same patient serves as both experiment and control, thereby negating differences between people such as smoking, age and gender.

Although it was not intended as part of the study and the study was not designed to evaluate ridge preservation per se, the subjective clinical observation at the time of harvesting and implant placement was however that the grafted sockets were better preserved in terms of the volume and "feel" of the bone – confirmed by the results of various studies [1, 2, 5, 6, 10, 12 13, 15]. This phenomenon greatly facilitates the placement of implants without the need for secondary augmentation procedures and is possibly the main reason why so many clinicians routinely perform socket grafting at the time of extraction, justifying the additional clinical intervention and patient discomfort as well as the increased financial implications.

It should also be noted that the primary researcher is not a trained histopathologist, but was throughout advised by a highly trained oral pathologist and supervised by an

experienced periodontist. Reasonable intra and inter-rater agreement was achieved, ranging from “good” agreement for inflammatory cells and blood vessels and “excellent” agreement for the other indicators [29]. It should be noted that there were one or two blinded recounts, under instruction of the co-supervisor as statistician, by both the primary researcher and research supervisor to achieve adequate inter-rater agreement. This requirement could probably be contributed to initial data capturing errors in a very big data set.

Overall, the results obtained was considered accurate enough to draw the following inference.

Chapter 7

Conclusion

This study compared bone quality of naturally healing sockets to sockets grafted with DFDBA. Histologically, mainly by assessing osteocyte counts, percentage of trabecular bone formation and percentage of collagen/connective tissue, no real statistical differences could be found between the grafted and non-grafted sites.

These findings therefore tend to support the null hypothesis that “DFDBA does not improve the quality of bone in extraction sockets”. However, the small sample size limits the findings of this study – yet the small differences observed between groups may warrant further studies.

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Appendix A

Consent letter from the CEO of UPOHC

Chairperson:
Prof LM Sykes
Members:
Prof T Swart
Prof SM Dawjee
Dr P Brandt
Prof A Bhayat
Secretary:
Ms C Swart

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Prof AJ Ligthelm

Dean

School of Dentistry

2016-05-18

Dear Professor

PROTOCOL APPROVAL: DENT 2016/18

Name: Dr JPJ Olivier

Title: "Histologic healing following tooth extraction with socket grafting using demineralised freeze-dried bone allograft (DFDBA), compared to undisturbed normal healing in humans: a randomised controlled trial."

The protocol attached hereto was evaluated by the Research Committee of the School of Dentistry. The Research Committee recommends the approval of the title and the protocol.

Yours sincerely

LM Sykes

PROF L SYKES

CHAIRPERSON: RESEARCH COMMITTEE

Protocol approved/not approved

(Acting Chairperson/CEO)

PROF AJ LIGTHELM

DEAN: SCHOOL OF DENTISTRY

26/5/16.

Appendix B

Consent letter from the Ethics Committee – 1

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.
- FWA 00002567, Approved dd 22 May 2002 and Expires 20 Oct 2016.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Expires 22/04/2017.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences Research Ethics Committee

30/06/2016

Approval Certificate
New Application

Ethics Reference No.: 231/2016

Title: HISTOLOGIC HEALING FOLLOWING TOOTH EXTRACTION WITH SOCKET GRAFTING USING DEMINERALISED FREEZE-DRIED BONE ALLOGRAFT (DFDBA), COMPARED TO UNDISTURBED NORMAL HEALING IN HUMANS: A RANDOMISED CONTROLLED CLINICAL TRIAL

Dear Johannes Petrus Jacobus Olivier

The **New Application** as supported by documents specified in your cover letter dated 27/06/2016 for your research received on the 27/06/2016, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 29/06/2016.

Please note the following about your ethics approval:
- Ethics Approval is valid for 2 years
- Please remember to use your protocol number (**231/2016**) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, or monitor the conduct of your research.

Ethics approval is subject to the following:
- The ethics approval is conditional on the receipt of **6 monthly written Progress Reports**, and
- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

We wish you the best with your research.

Yours sincerely

*** Kindly collect your original signed approval certificate from our offices, Faculty of Health Sciences, Research Ethics Committee, Tswelopele Building, Level 4-59*

Dr R Sommers; MBChB; MMed (Int); MPharMed,PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes 2004 (Department of Health).

☎ 012 356 3085 fhsethics@up.ac.za http://www.up.ac.za/healthethics
✉ Private Bag X323, Arcadia, 0007 - Tswelopele Building, Level 4-59, Gezina, Pretoria

Appendix C

Consent letter from the Ethics Committee – 2

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Health Sciences

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria complies with ICH-GCP guidelines and has US Federal wide Assurance.

- FWA 00002567, Approved dd 22 May 2002 and Expires 03/20/2022.
- IRB 0000 2235 IORG0001762 Approved dd 22/04/2014 and Exoires 03/14/2020.

18 June 2019

Approval Certificate
Amendment

Ethics Reference No.: 231/2016
Title: HISTOLOGIC HEALING FOLLOWING TOOTH EXTRACTION WITH SOCKET GRAFTING USING DEMINERALISED FREEZE-DRIED BONE ALLOGRAFT (DFDBA), COMPARED TO UNDISTURBED NORMAL HEALING IN HUMANS:

A RANDOMISED CONTROLLED CLINICAL TRIAL

Dear Dr JPJ Olivier

The **Amendment** as supported by documents received between 2019-05-28 and 2019-06-18 for your research, was approved by the Faculty of Health Sciences Research Ethics Committee on its quorate meeting of 2019-06-12.

Please note the following about your ethics approval:

- Please remember to use your protocol number (231/2016) on any documents or correspondence with the Research Ethics Committee regarding your research.
- Please note that the Research Ethics Committee may ask further questions, seek additional information, require further modification, monitor the conduct of your research, or suspend or withdraw ethics approval.

Ethics approval is subject to the following:

- The ethics approval is conditional on the research being conducted as stipulated by the details of all documents submitted to the Committee. In the event that a further need arises to change who the investigators are, the methods or any other aspect, such changes must be submitted as an Amendment for approval by the Committee.

Decision:

Recommendations of Prelim meeting 15 May 2019
•□We recommend that the following comments be addressed:
1. Please submit an amendment for these changes, with necessary documents.
•□Please submit a cover letter (point by point) indicating all revision/s made together amended documents.

We wish you the best with your research.

Yours sincerely

Dr R Sommers

Research Ethics Committee
Room 4-60, Level 4, Tswelopele Building
University of Pretoria, Private Bag X323
Arcadia 0007, South Africa
Tel +27 (0)12 356 3084
Email deepeka.behari@up.ac.za
www.up.ac.za

Fakulteit Gesondheidswetenskappe
Lefapha la Disaense tša Maphelo

MBChB MMed (Int) MPharmMed PhD
Deputy Chairperson of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

The Faculty of Health Sciences Research Ethics Committee complies with the SA National Act 61 of 2003 as it pertains to health research and the United States Code of Federal Regulations Title 45 and 46. This committee abides by the ethical norms and principles for research, established by the Declaration of Helsinki, the South African Medical Research Council Guidelines as well as the Guidelines for Ethical Research: Principles Structures and Processes, Second Edition 2015 (Department of Health).

Research Ethics Committee
Room 4-60, Level 4, Tswelopele Building
University of Pretoria, Private Bag X323
Arcadia 0007, South Africa
Tel +27 (0)12 356 3084
Email deepeka.behari@up.ac.za
www.up.ac.za

Appendix D

Declaration by investigator

RESCOM C

COMMITMENTS AND RESPONSIBILITIES OF SUB- INVESTIGATORS REQUIRED FOR RESEARCH THROUGH THE FACULTY OF HEALTH SCIENCES RESEARCH ETHICS COMMITTEE, UNIVERSITY OF PRETORIA

DECLARATION BY INVESTIGATOR:

I agree to **personally** conduct or supervise the described investigation.

I understand as sub-investigator that I am **totally responsible** for aspects of the study delegated to me by the Principal Investigator and am legally bound by the contract signed with the sponsor and **will not inappropriately delegate my responsibilities** to the rest of my study team.

I have **read and understand the information in the investigator's brochure**, including the potential risks and side effects of the drug.

I agree **to ensure** that all associates, colleagues, and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments, without relinquishing my total responsibility for the study.

I confirm that I am **suitably qualified and experienced** to perform and/or supervise the study proposed.

I agree to conduct the study in accordance with the relevant, current protocol and will only make changes in the protocol after approval by the sponsor and the Ethics Committee, except when urgently necessary to protect the safety, rights, or welfare of subjects.

I agree to inform any patients, or any persons used as controls, that the drugs are being used for investigational purposes and **I will ensure that the ICH GCP Guidelines and Ethics Committee requirements relating to obtaining informed consent are met.**

I agree to timeously report to the sponsor and Ethics Committee adverse experiences that occur in the course of the investigation according to the time requirements adopted by the Faculty of Health Sciences Research Ethics Committee, University of Pretoria.

I agree to maintain **adequate and accurate** records and to make those records available for inspection by the appropriate authorized agents, be it EC, FDA or sponsor agents.

I agree to comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements in the Declaration of Helsinki and South African and ICH GCP Guidelines and am conversant with these guidelines.

I agree to inform the Ethics Committee in advance should I go on leave together with an agreed plan of action regarding an alternate principal investigator or sub-investigator to take responsibility in my absence.

I understand that the study may be audited at any time and that deviation from the principles in this declaration will be put before the Ethics Committee for action, which may include disqualification as an investigator and rehabilitation before being accepted as an investigator in other studies.

I confirm that there is no conflict of interest whatsoever in my participation in this study. I have no shares in the sponsoring company and my participation and interests are as defined in the financial agreement.

SIGNATURE OF SUB-INVESTIGATOR	NAME (Printed) PROF. AW VAN ZYL	DATE 3/5/2016
SIGNATURE OF PRINCIPAL INVESTIGATOR	NAME (Printed) JRS OLIVIER	DATE 2016/05/02

RESCOM C

Appendix E

Letter of clearance from biostatistician

LETTER OF CLEARANCE FOR STATISTICS

HISTOLOGIC HEALING FOLLOWING TOOTH EXTRACTION WITH SOCKET GRAFTING USING DEMINERALISED FREEZE-DRIED BONE ALLOGRAFT (DFDBA), COMPARED TO UNDISTURBED NORMAL HEALING IN HUMANS: A RANDOMISED CONTROLLED CLINICAL TRAIL

For the degree

MScDent

Author: JPJ Olivier

I hereby confirm that I am aware of the project and will assist with the statistical analysis of the data generated from the project.

The DATA ANALYIS will consist of descriptive statistics and paired t-tests or appropriate non-parametric equivalent.

Sample size

- 10 patients with paired data will have 80% power to detect a difference of one standard deviation.

Name _____ **Prof TC Postma** _____

Signature _____ _____

Date: _____ **Wednesday 22 May 2019** _____

Appendix F

Patient information and informed consent

Updated 23/09/2014

PICD 1 (a)

PATIENT / PARTICIPANT'S INFORMATION & INFORMED CONSENT DOCUMENT

(Each patient must receive, read and understand this document before the start of the study)

If a child is 18 years or younger and is requested to partake in a research study, the parent /legal guardian must give consent. However children from 7-18 years must also sign an ASSENT FORM (This form must be written in layman's language/terms to enable a grade 5 learner to understand.)

TRIAL TITLE: HISTOLOGIC HEALING FOLLOWING TOOTH EXTRACTION WITH SOCKET PRESERVATION USING DFDBA COMPARED TO UNDISTURBED NORMAL HEALING IN HUMANS: A RANDOMISED CONTROLLED CLINICAL TRAIL

SPONSOR:

Principal Investigators: JPJ Olivier

Institution: Private Practise / University of Pretoria

DAYTIME AND AFTER HOURS TELEPHONE NUMBER(S):

Daytime numbers: 011/898-6517

After hours: 083 508 3688

DATE AND TIME OF FIRST INFORMED CONSENT DISCUSSION:

			:	
dd	mmm	ivy	Time	

ADDENDUM A

PATIENT / PARTICIPANT'S INFORMATION LEAFLET & INFORMED CONSENT FORM FOR CLINICAL RESEARCH

Dear Patient

Welcome to this facility. Below please find information about a research study we are conducting on extraction socket preservation. I, Dr Jan Olivier, will be conducting this study and would like to invite you to volunteer for this research project. Please read this information leaflet carefully and should you decide to participate, I will explain the study in detail and answer any questions you or your family members may have. Patients participating in this study may perhaps not benefit directly from the participation, but information gained from their participation will potentially benefit future dental implant patients by increasing our understanding of the complexity of socket healing, thus enabling better management of such patients in future.

TRIAL TITLE: HISTOLOGIC HEALING FOLLOWING TOOTH EXTRACTION WITH SOCKET GRAFTING USING DEMINERALIZED FREEZE-DRIED BONE ALLOGRAFT (DFDBA), COMPARED TO UNDISTURBED NORMAL HEALING IN HUMANS: A RANDOMISED CONTROLLED CLINICAL TRAIL

INTRODUCTION

This information leaflet is to help you to decide if you would like to participate. Before you agree to take part in this study you should fully understand what is involved. If you have any questions, which are not fully explained in this leaflet, do not hesitate to ask the investigator. You should not agree to take part unless you are completely happy about all the procedures involved. In the best interests of your health you are most welcome to discuss with or inform your personal doctor of your possible participation in this study, wherever possible. I will be notifying your personal doctor in this regard should you wish me to do so.

WHAT IS THE PURPOSE OF THIS STUDY?

With implant provision becoming more widespread, the need to anticipate and pre-empt bone loss after extraction has become an ever increasing concern among clinicians. Inserting foreign materials could however produce potential added risks of post-operative complications such as pain, discomfort and the very rarely documented possibility of disease transmission, as well as greater cost to the patient - with possibly no added value or justification for subsequent restorative treatment. Currently, there is no consensus on building up of an extraction socket with DFDBA ("Bone Sugar") immediately after extraction of a tooth. The aim of this study is to assess microscopically whether such grafting could be advantageous or not.

WHAT IS THE DURATION OF THIS TRIAL?

If you decide to take part you will be one of approximately 10 patients. The study will last for up to 20 weeks. You will be asked to visit the investigator 8 times as per the following schedule: sutures to be removed after 10 days and follow-up appointments every two weeks until implant placement 16 - 20 weeks after removal of teeth.

DESCRIPTION OF PROCEDURES TO BE FOLLOWED

It is important that you let the investigator know of any medicines (both prescriptions and over-the-counter medicines), alcohol or other substances that you are currently taking.

- Intra-oral examination, radiological and CBCT (Cone Beam Computerised Tomography) scans, together with an ITI (International Team for Implantology) classification, will be performed pre-operatively on each patient.
- Customised acrylic guides to be fabricated on pre-extraction study models to serve as fixed reference guides for accurate harvesting of samples and subsequent placement of implants.
- The relevant teeth will be removed with as little trauma as possible to try and ensure preservation of the socket walls. One socket will be left to heal normally, but with the other a full-thickness flap will be raised to expose both labial and lingual/palatal aspects of the alveolar ridge before commencement of tooth removal. After removal the DFDBA will be inserted into the socket and a collagen membrane (Jason Membrane – Botiss Biomaterials) will be placed to completely cover the socket extending a minimum of 3mm beyond the alveolar crest, whereafter the flap will be replaced and sutured with non-resorbable sutures.
- Post-operatively all patients will receive appropriate antibiotic cover, Chlorhexidine mouth wash twice daily for 10 days as well as pain and anti-inflammatory medication.
- Sutures to be removed after 10 days and patients followed-up every two weeks until implant placement 16 - 20 weeks after removal of teeth.
- At 16 – 20 weeks core samples of at least 8mm in length are to be harvested by means of a hollow drill as the first step during the process of implant placement.

HAS THE STUDY RECEIVED ETHICAL APPROVAL?

This clinical trial Protocol was submitted to the Faculty of Health Sciences Research Ethics Committee, University of Pretoria, telephone numbers 012 3541677 / 012 3541330, and written approval has been granted by that committee. The study has been structured in accordance with the Declaration of Helsinki (last update: October 2000), which deals with the recommendations guiding doctors in biomedical research involving human/subjects. A copy of the Declaration may be obtained from the investigator should you wish to review it.

WHAT ARE YOUR RIGHTS AS A PARTICIPANT IN THIS STUDY?
Your participation in this trial is entirely voluntary and you can refuse to participate or stop at any time without stating any reason. Your withdrawal will not affect your access to other dental care. The investigator retains the right to withdraw you from the study if it is considered to be in your best interest. If it is detected that you did not give an accurate history or did nor follow the guidelines of the trial and the regulations of the trial facility, you may be withdrawn from the trial at any time.

MAY ANY OF THESE PROCEDURES RESULT IN DISCOMFORT OR INCONVENIENCE?
As with any minor oral surgical procedures one should anticipate some degree of discomfort, pain and possibly some swelling. To alleviate such symptoms and reduce the risk of infection patients will be required to take medication according to the pre-determined protocol i.e. antibiotics, painkiller/anti-inflammatory combination and a mouthwash. Stitches that are placed will have to be removed after 10 days.

WHAT ARE THE BENEFITS TO YOU
The investigator will arrange funding, by means of sponsorships, for all procedures and reasonable medical expenses which you may incur as a direct result of this study as determined by the University of Pretoria and the investigator.

WHAT ARE THE RISKS INVOLVED IN THIS STUDY?
No risks other than the normal risk that goes with minor oral surgical procedures, such as extractions and placing of implants, will be experienced, i.e. there will be some discomfort and possibly some swelling after undergoing the procedure and taking of the samples as explained above. Your protection is that the procedures are performed under sterile conditions by experienced personnel. However, should unexpected complications arise in any of the sites, such complications will be dealt with as a matter of priority even if it means abandonment of the trial.

ARE THERE ANY WARNINGS OR RESTRICTIONS CONCERNING MY PARTICIPATION IN THIS STUDY?
No. Should you be on blood thinning medication (such as aspirin), we will liaise with your personal doctor to withdraw the medication or to make alternative arrangements.

INSURANCE AND FINANCIAL ARRANGEMENTS
You will not be paid to participate in this trial and costs for the restorative phase, such as crowns or dentures, will be for the account of the patient.

SOURCE OF ADDITIONAL INFORMATION
For the duration of the study you will be under the care of Dr Jan Olivier. If at any time you feel that you have any symptoms that are causing you problems, or if you have any questions regarding the study and treatment, please do not hesitate to contact him. The telephone number through which you can reach him is 011/898-6517, or after hours he can be reached on his cellular phone: 083 508 3688.

CONFIDENTIALITY

All information obtained during the course of this study is strictly confidential. Data that may be reported in scientific journals will not include any information which identifies you as a patient in this study. Any information uncovered regarding your state of health as a result of your participation in this trial will be held in strict confidence. You will be informed of any finding of importance to your health or continued participation in this study, but this information will not be disclosed to any third party in addition to the ones mentioned above without your written permission.

INFORMED CONSENT

I hereby confirm that I have been informed by the investigator, Dr Jan Olivier, about the nature, conduct, benefits and risks of this study. I have also received, read and understood the above written information (Patient Information Leaflet and Informed Consent) regarding the clinical trial.

I am aware that the results of the study, including personal details regarding my gender, age, date of birth, initials and diagnosis will be anonymously processed into a study report.

I may, at any stage, without prejudice, withdraw my consent and participation in the study. I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.

Patient's name:
(Please print)

Patient's signature:

Date:

I, Dr Jan Olivier, herewith confirm that the above patient has been informed fully about the nature, conduct and risks of the above study.

Investigator's name: Dr Jan Olivier

Investigator's signature:

Date:

Witness's name:
(Please print)

Witness's signature:

Date:

*Consent procedure should be witnessed whenever possible.

VERBAL PATIENT INFORMED CONSENT (Applicable when patients cannot read or write)

I, the undersigned, Dr Jan Olivier, have read and have explained fully to the patient, named …… and/or his/her relative, the patient information leaflet, which has indicated the nature and purpose of the study in which I have asked the patient to participate. The explanation I have given has mentioned both the possible risks and benefits of the study. The patient indicated that he/she understands that he/she will be free to withdraw from the study at any time for any reason.

I hereby certify that the patient has agreed to participate in this study.

Patient's Name:
(Please print)

Investigator's Name: Dr Jan Olivier
(Please print)

Investigator's Signature:

Date:

Witness's Name:
(Please print)

Witness's Signature:

Date:

(Witness - Sign that he/she has witnessed the process of informed consent)

Appendix G

Declaration of Helsinki

WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964
and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words,

"The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by

individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and

standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain

for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention

and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made

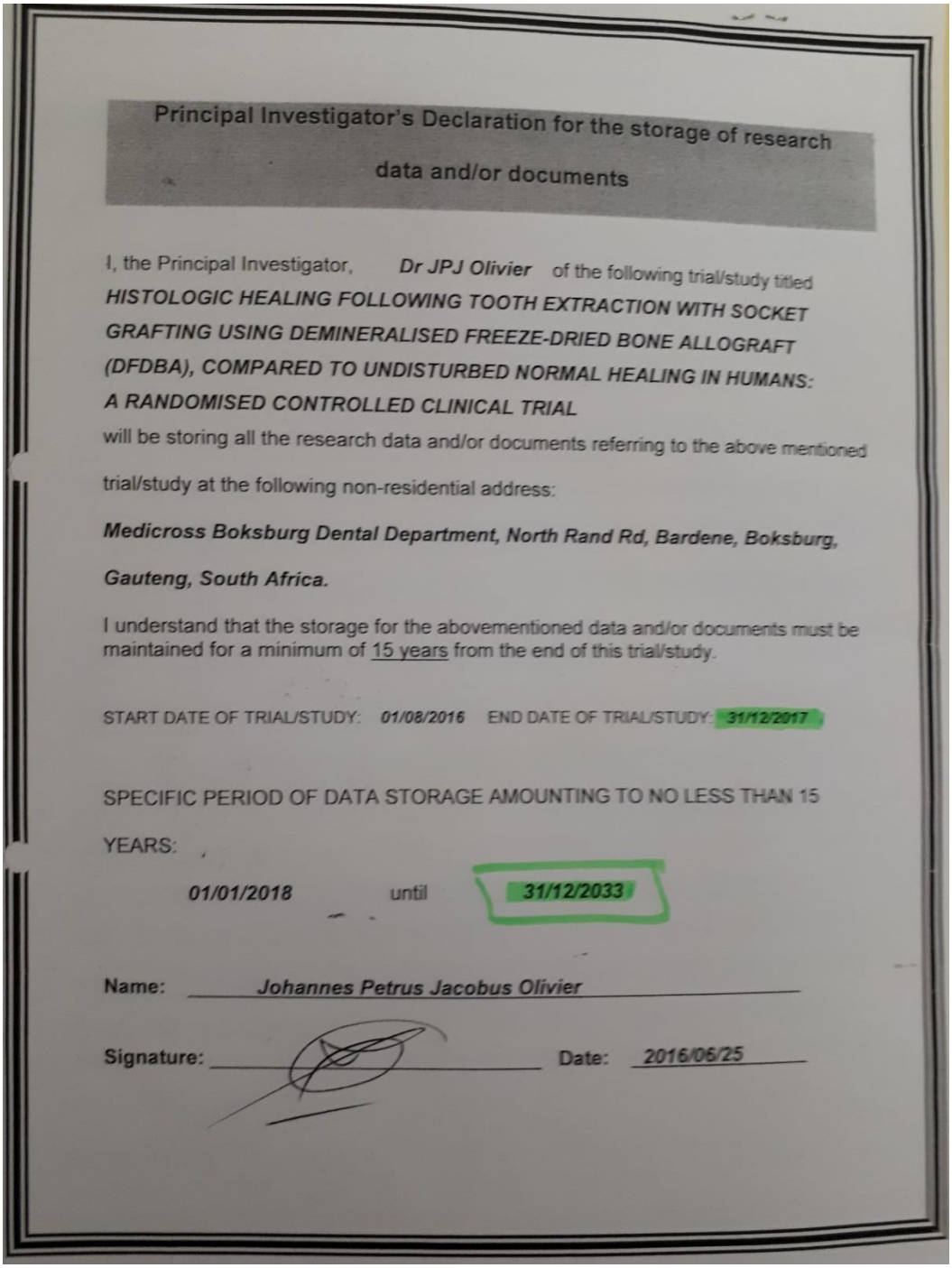
publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

Appendix H

Data storage form



Principal Investigator's Declaration for the storage of research data and/or documents

I, the Principal Investigator, ***Dr JPJ Olivier*** of the following trial/study titled ***HISTOLOGIC HEALING FOLLOWING TOOTH EXTRACTION WITH SOCKET GRAFTING USING DEMINERALISED FREEZE-DRIED BONE ALLOGRAFT (DFDBA), COMPARED TO UNDISTURBED NORMAL HEALING IN HUMANS: A RANDOMISED CONTROLLED CLINICAL TRIAL*** will be storing all the research data and/or documents referring to the above mentioned trial/study at the following non-residential address:

Medicross Boksburg Dental Department, North Rand Rd, Bardene, Boksburg, Gauteng, South Africa.

I understand that the storage for the abovementioned data and/or documents must be maintained for a minimum of 15 years from the end of this trial/study.

START DATE OF TRIAL/STUDY: ***01/08/2016*** END DATE OF TRIAL/STUDY: ***31/12/2017***

SPECIFIC PERIOD OF DATA STORAGE AMOUNTING TO NO LESS THAN 15 YEARS:

01/01/2018 until ***31/12/2033***

Name: ***Johannes Petrus Jacobus Olivier***

Signature: _____ Date: ***2016/06/25***