# PERIODONTAL REGENERATION OF 1-, 2-, AND 3-WALLED INTRABONY DEFECTS USING ACCELL CONNEXUS VERSUS DEMINERALIZED FREEZE-DRIED BONE ALLOGRAFT: A RANDOMIZED PARALLEL ARM CLINICAL CONTROL TRIAL

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A thesis submitted to the Faculty of the
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in partial fulfillment of the requirements for the degree of
Master of Science
in Oral Biology

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CERTIFICATE OF APPROVAL

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MASTER'S THESIS

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This is to certify that the Master's thesis of

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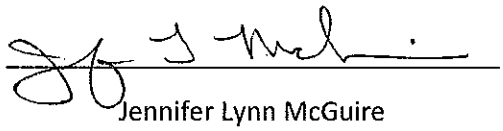

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ABSTRACT

PERIODONTAL REGENERATION OF 1-, 2-, AND 3-WALLED INTRABONY DEFECTS USING ACCELL CONNEXUS VERSUS DEMINERALIZED FREEZE-DRIED BONE ALLOGRAFT: A RANDOMIZED PARALLEL ARM CLINICAL CONTROL TRIAL

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Certificate in Periodontics, Periodontics Department, 2013

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Introduction: This thesis discusses the research project undertaken to determine if Accell connexus, a demineralized freeze-dried bone allograft product that contains 5-7 times the amount of bone morphogenetic proteins as regular demineralized freeze-dried bone allograft (DFDBA) provides superior periodontal regeneration (formation of new bone, cementum, and connective tissue around teeth) than regular demineralized freeze-dried bone allograft.

Methods and Materials: Thirty patients diagnosed with severe periodontitis, having at least one intrabony defect with a probing depth $\geq$ 6 mm, will be enrolled. Participants will have impressions made of their upper and lower teeth to provide dental stone models of the maxillary and mandibular arches. Customized plastic stents fabricated on the models will be used by blinded investigators to obtain standardized clinical measurements of the defects before surgery and at 6 and 12 months after surgery. Measurements will include probing depths and clinical attachment levels. Standardized digital radiographs using a customized bite-plate and a paralleling technique for reproducibility will be used to take periapical radiographs at baseline and 6 months and 12 months after surgery. All participants will receive the same standardized surgical approach. Whether surgery is performed with local anesthesia only, or under local anesthesia and sedation, will be a choice made between the surgeon and the patient. During surgery, the depth and width

of the intrabony defect will be measured after the defect is debrided. Study investigators (not the participant's surgeon) blinded to the bone graft material a participant receives will make all clinical measurements and take radiographs for the study. After defect debridement, and before diseased root surface treatment with ethylenediaminetetraacetic acid (EDTA), the surgical team will be handed a sealed envelope containing the name of the bone graft material that the participant was randomized to receive: DFDBA or Accell. Fifteen patients will receive DFDBA and fifteen patients receive Accell. Participants will be re-evaluated to assess postoperative healing at weeks 1, 2, 4, 6, 8, 12, and 16, and again at 6, 9 and 12 months. After 12 months the protocol is complete and participants will pursue maintenance therapy with their surgeons. Data analysis will compare changes in clinical attachment levels, probing depths and radiographic bone levels. These pre-surgical and post-surgical assessments will use the Mann-Whitney U test.

Results: There are no results to report at this time. Patients are currently being enrolled. At present, two patients have been enrolled in the study. The surgical procedure has been completed on one patient. Six month data for this patient will be collected in July 2013.

Discussion/Conclusion: At present since we do not have any results to present, no conclusions can be made. Based upon clinical experience using the material Accell connexus, we predict the Accell connexus will show improved bone fill and clinical attachment levels compared to demineralized freeze-dried bone allograft.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| BMP | Bone Morphogenetic Protein(s) |
| CAL | Clinical Attachment Level |
| CEJ | Cementoenamel Junction |
| DFDBA | Demineralized Freeze-Dried Bone Allograft |
| FDBA | Freeze-Dried Bone Allograft |
| GS | Gingival Sulcus |
| JE | Junctional Epithelium |
| OE | Oral Epithelium |
| PD | Probing Depth |
| PDL | Periodontal Ligament |
| SE | Sulcular Epithelium |

## INTRODUCTION

The goal of periodontal therapy is preservation of the natural dentition by controlling the inflammatory processes that cause bone loss and eventual tooth loss. When oral hygiene and non-surgical therapies cannot control periodontitis surgical therapy is advised to clean deep pockets and try to create a periodontal architecture for efficient plaque control to minimize recurrence of inflammation. More than half a century ago pocket reduction surgery via osseous re-contouring of diseased bone was the primary approach, but often resulted in un-esthetic and uncomfortable root exposure. As wound healing and etiology became better understood the field moved towards regenerative therapy, aimed at re-growing loss periodontal structures to improve post-surgical architecture. For the last 40 years, the best material for periodontal regenerative therapy has been demineralized freeze-dried bone allograft (DFDBA). The demineralization process for DFDBA unmasks bone morphogenetic proteins (BMP) that induce bone formation, but DFDBA's particulate nature makes it difficult to be retained in periodontal defects that lack bony walls. Complete restoration with DFDBA of extensive defects to pre-disease periodontal anatomy is frequently not possible. Although periodontal support is enhanced, many patients still require frequent visits for maintenance therapy to avoid further breakdown, and minimize sudden abscess formation in residual pockets. Although numerous products containing various growth factors have come to market, they have tended to be very expensive and not necessarily more efficacious than traditional DFDBA. Recently, the FDA had approved Accell connexus. It is a DFDBA preparation that contains 5 to7 times more BMP than traditional preparations and has a putty-like consistency that allows it to stay in place when applied to large periodontal defects that lack walls. DFDBA has been the gold standard for periodontal regenerative therapy for decades, but Accell has characteristics that address inherent weaknesses in the gold standard. Initial clinical observations suggest that Accell may be a significant step forward in regenerative therapy, but no study has directly compared traditional DFDBA to Accell. In the military setting improved regenerative therapy would reduce the frequency of periodontal maintenance visits and minimize susceptibility to events like abscesses that can pose medical issues to soldiers and sailors during deployment.

## REVIEW OF THE LITERATURE

### INTRODUCTION

The periodontium refers to the tissues that support teeth in the jaws (the maxilla and the mandible). As seen in the adjacent diagram, the periodontium consists of the gingiva (gum tissue), the periodontal ligament (PDL), cementum that covers a tooth's root and the jaw's alveolar bone (Newman 06). The gingiva is composed of epithelium and supracrestal connective tissue fibers oriented above the bone and PDL, which forms an epithelium-lined sulcus or crevice that encircles teeth (Pöllänen 2003). The PDL collagen fibers span the space between the alveolar bone and tooth root and suspend teeth in the jaw by their insertions into the cementum and alveolar bone.

One of the best series of illustrations that depict the periodontium in health and disease is the Page and Schroeder Model of Pathogenesis (1976) with images for health, gingivitis and periodontitis. Three types of epithelia tissues characterize the gingiva: (1) the keratinized oral epithelium (OE) that comprises the visible band of gingiva around the teeth, (2) the sulcular epithelium (SE) which is the transition tissue at the edge of the tooth, and (3) the specialized non-keratinized epithelium called the junctional epithelium (JE) that lines the bottom of the gingival sulcus (GS). The JE adheres to teeth and in health serves as the first barrier to prevent bacterial plaque from reaching the underlying connective tissue and bone (Shimono 2003).

The terms gingival sulcus (GS) and gingival crevice are used interchangeably. Historically, sulcus was reserved to describe health while crevice was used when inflammation altered gingival architecture. The above image depicting "Health" shows how the intact JE, supracrestal fibers (CO) and the PDL between the alveolar bone and root surface support a tooth. The PDL fibers serve as the connective tissue support that holds the tooth in place in the bone and helps cushion the tooth from forces when we bite, chew or clench (Beersten 97). The PDL contains pressure receptors that are activated by tooth contact. The impulses generated by these receptors are sent to the brain and used to help coordinate the sequencing of jaw movement (Byers 89).

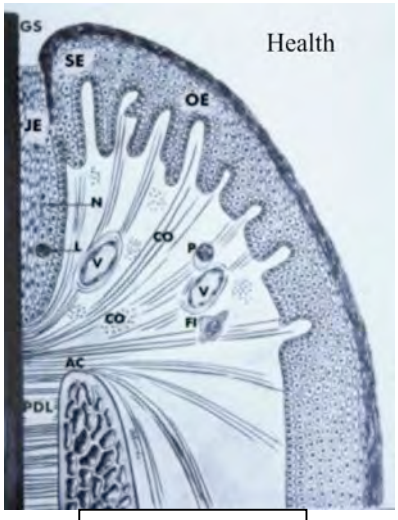


Fig 1: Health

Bacterial plaque is always forming. If it is allowed to persist in the GS it can induce gingival inflammation. As is visible in Page and Schroeder "Gingivitis" diagram the integrity of JE is disturbed as inflammatory cells accumulate in the underlying connective tissue. Gingivitis is the early form of periodontal disease and while the gums can become red, swollen and sore with irritation of supracrestal gingival fibers, the PDL attachment and the alveolar bone remain intact.

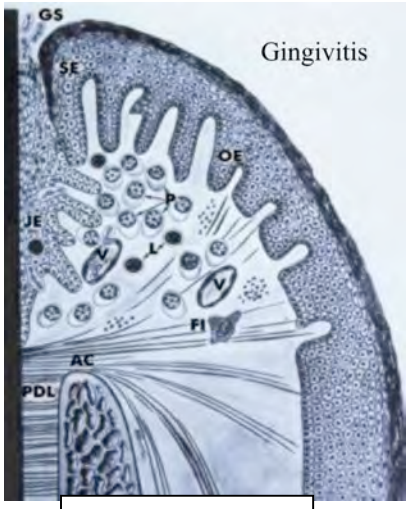


Fig 2: Gingivitis

Gingivitis transitions to periodontitis when the host's immune response cannot resist bacterial plaque and the inflammatory process (Weinman 1941; Takata 1988). Connective tissue and alveolar bone destruction leading to possible tooth loss is a function of the interaction between the plaque front and immune response (Haffajee 1983; Waerhaug 1977).

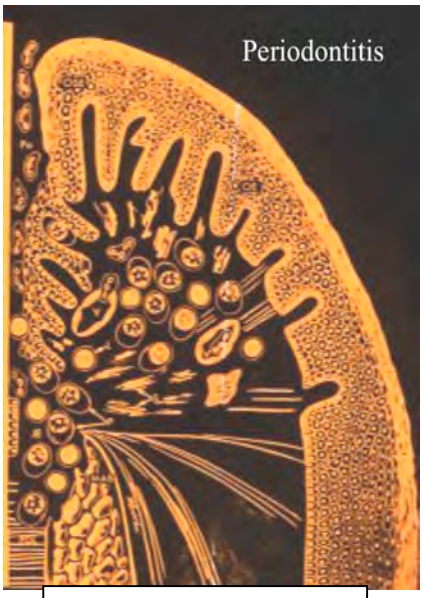


Fig3: Periodontitis

This tissue breakdown is clear in the Page and Schroeder "Periodontitis" diagram. With the onset of periodontitis, the gingival crevice becomes a deeper periodontal pocket with destruction of supracrestal gingival fibers and PDL, and the breakdown of the alveolar bone. This phenomenon of connective tissue and bone destruction is referred to as clinical attachment loss.

Clinical attachment loss is a measure of how much periodontal support has been destroyed. It is assessed by gently inserting a periodontal probe into the GS or a periodontal pocket to measure probing depth (PD). These probing depths are recorded on a periodontal chart. Periodontal probes have 1 or 2 mm increments up to 15mm etched on their surfaces. In health, insertion of the periodontal probe is resisted by the intact connective tissue and probing depths will measure 3mm or less. When periodontal disease is present, the periodontal probe inserts more deeply into the pocket because the connective tissue has been destroyed. In a diseased state, the probing depths will be 4mm or greater, and they bleed upon probing (Armitage 77). Probing depth measurements aid in the diagnosis of the severity of the periodontal disease. The adjacent illustration shows a periodontal probe inserted into a

healthy GS on the left and a probe being inserted into moderately deep pocket on the right. It is not uncommon to have relatively healthy and significantly diseased locations on the same tooth.

As the disease process continues and more connective tissue is lost, bone loss becomes evident on radiographs once sufficient mineral content in the bone has been destroyed. The radiograph provides a visual tool for detecting and characterizing intrabony defects (Rees 71). However, clinical detection of attachment loss indicated by deeper probing depths usually precedes radiographic evidence of bone loss by a period of 6-8 months (Goodson and Haffajee 84). Probing depths recorded on a detailed chart and radiographs help clinicians assess periodontal disease severity (localized or generalized) and plan appropriate treatment. Radiographs help determine whether bone loss is horizontal, vertical, or a combination of the two. Regeneration of bone is more predictable when bone loss is vertical, meaning that the bone loss is a void or hole with some bony walls remaining (Quintero 82, Cortellini 93). Bony walls help retain bone graft material, and the more walls the better. Horizontal bone loss means no walls are remaining. The adjacent clinical image shows a 3-walled intrabony defect that has been debrided and is ready to receive bone graft material. The soft tissue flaps that were elevated to expose the defect will be re-approximated around the teeth after bone graft placement. Three walled defects are best suited for holding the bone graft in place and offer the greatest chance for maximum regeneration of tissues destroyed by periodontitis (Cortellini 93).

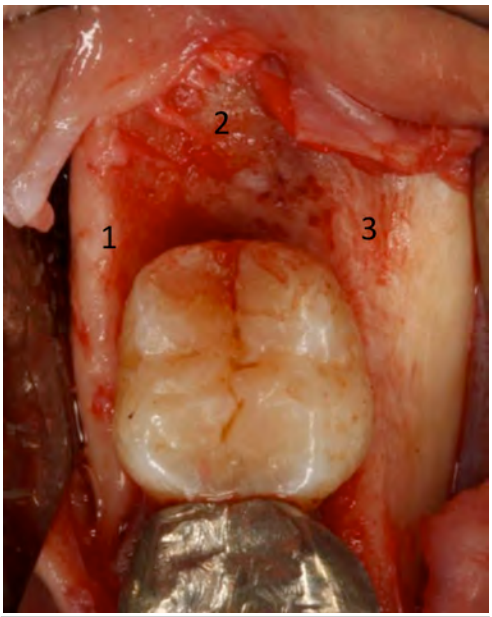



Fig 4: Example of a 3-walled intrabony defect

**PERIODONTAL TREATMENT: SEQUENCING**

Oral Hygiene

After diagnosis, patients are taught specific oral hygiene procedures; how to best prevent plaque accumulation because therapies fail if adequate plaque control is not maintained. Since proper flossing can only disorganize immature plaque in a 3 mm deep gingival sulcus, and tooth brushing can only effectively clean 0.5-1 mm deep into the sulcus (Waerhaug 78), improving periodontal tissue architecture (gaining attachment by decreasing probing depths) for efficient oral hygiene is a primary therapeutic goal.

Initial Therapy

The beginning of treatment is called initial therapy. Clinicians attempt to remove the products of plaque accumulation in pockets and on teeth by non-surgical scaling and root planing. This is commonly known as a "deep cleaning." While effective at removing calculus (hardened plaque) and bacteria in relatively shallow probing depths, the efficacy of scaling and root planing begins to decline as probing depths deepen beyond 3.73mm (Stambaugh 81). In periodontal disease, pocket depths are >4mm, meaning that the root surfaces in deeper pockets cannot be predictably cleaned of plaque and calculus. When a patient has probing depths greater than 4 mm after initial therapy, surgical treatment is often indicated in order to gain access to adequately debride the diseased root surfaces and pockets so that healing can occur.

Surgery

Surgical therapy for periodontal disease is classified as resective or regenerative. Although each surgical approach elevates soft tissue flaps to provide access for debridement of calculus and plaque from roots and pockets, the goal to create a resulting periodontal architecture that helps with proper hygiene is pursued by different techniques. Resective surgery involves removing some soft tissue and non-supportive alveolar bone around the teeth to hopefully "reshape" the soft tissue (gums) architecture more favorable for plaque control (Selipsky 76).

On the other hand, regenerative therapy does not resect soft tissue or bone from around the tooth. Rather, after cleaning the diseased root surfaces, the roots are treated and the bony defects are filled with bone particles in the hope to regenerate bone, cementum, and periodontal ligament that had been destroyed by the disease process. To achieve regeneration of periodontal tissue components, it is necessary during post-surgical healing to inhibit the downward growth of the gingival epithelium from the flap margins onto the cleaned root surface (Melcher 76). Since epithelium grows much faster than connective tissue, exclusion of the gingival epithelium is essential in order to provide necessary time for the bone cells to make bone and fibroblasts to make new collagen so new PDL fibers can insert into new cementum on the root. Bone grafts particles added to the defect help keep the epithelium away from the tooth margin. A membrane, a thin material placed over the defect site, can also be used as a barrier to inhibit downward growth of gingival epithelium. A combination of bone particles and membrane is an effective way to regenerate lost periodontal tissues in intrabony defects (Guillemin 93, Shallhorn 88) when combined with thorough root preparation.

Root Preparation

Root preparation is completed prior to placement of the bone graft or membrane in periodontal regenerative therapy. The process is defined as "use of instruments or chemicals on roots to eliminate irritants, prevent bacterial accumulation, and encourage wound healing" (AAP Glossary of Terms). In root preparation, the teeth have initially been mechanically cleaned by instruments to remove visible plaque and calculus, but because plaque and calculus become imbedded into microscopic resorptive lacunae (holes) in root surface, it is almost impossible to remove 100 percent of all the plaque, calculus and bacteria (Lafferty 93). Therefore, mechanical debridement is followed by chemical treatment, and the most common chemical used for root preparation is tetracycline. Tetracycline (250 mg tablet) is diluted in sterile saline to a concentration of 50mg/mL. Sterile cotton pellets are saturated with the diluted tetracycline solution and burnished (rubbed) on the root surface for a period of three-five minutes (Trombelli 95). The acidity of the tetracycline removes residual plaque products in resorptive lacunae, and exposes collagen fibrils on the root surface that can inhibit downward migration of epithelium. Another benefit offered by tetracycline root biomodification is that its antibacterial properties have substantivity (effects last for an extended period of time). It has been found the antibacterial properties of tetracycline last for two weeks after the procedure (Demirel 91). Due to its ability to remove the plaque debris remnants, alter a root surface for new collagen attachment and antibacterial substantivity, tetracycline is widely used in periodontal regenerative therapy. An alternative agent that can be used in root preparation is ethylenediaminetetraacetic acid (EDTA). EDTA is a chelating agent and is commonly used in dentistry to remove the smear layer, or thin layer of inorganic debris covering the tooth surface. EDTA has been shown in the periodontal literature to be an adequate alternative to tetracycline for root surface preparation (Gamal 03).

Bone Grafts

Following root preparation, the next step in periodontal regenerative therapy is the selection and placement of the bone graft material. The bone graft materials are classified as either autografts, allografts, xenografts, or alloplasts.

1. An autograft is defined as "tissue transferred from one position to another within the same individual" (AAP Glossary of Terms). To obtain an autograft, a second surgical site is required to gain donor tissue. This second site can be the hip or another area in the jaw like the chin. Additional morbidity is associated with autografts because of the second surgical site.

2. An allograft is defined as "A graft between genetically dissimilar members of the same species" (AAP Glossary of Terms). Allograft bone is donor bone. A donor is screened for high risk lifestyle behaviors and tested for HIV, syphilis, Hepatitis A, Hepatitis B, Hepatitis C, and bacterial infection. Lymph nodes are examined for changes due to infection or parasites, and finally an autopsy is done to rule out any carcinomas. Allograft bone is harvested within 12 hours after a donor is pronounced dead. A sterile technique is used to harvest the cortical bone (outer dense bone) from the long bones (femur, humerous, etc…). The harvested long bone is cut into particles measuring 500 micrometers to five millimeters in diameter. The particles are washed first in water and then in a 100% ethanol bath for one hour. The ethanol bath deactivates viruses and removes remaining fat from the particles. Following the ethanol bath, the particles are frozen in nitrogen for two weeks while the samples from the donor are analyzed. When the testing results come back clear, the particles are then freeze-dried and ground into smaller particle sizes (range 250-750 micrometers). The bone will either be left in its mineralized form (mineralized freeze dried bone allograft or FDBA) or will be demineralized in 0.6 N hydrochloric acid to remove the inorganic content on the outside of the bone particles (DFDBA). De-minerlization exposes non-collagenous proteins such as bone morphogenetic proteins (BMP) which facilitate deposition of new bone by stimulating stem cells in the area of the defect to become bone forming cells. The demineralized bone is then washed in a sodium phosphate buffer to neutralize the acid and again freeze-dried. The mineralized and demineralized freeze-dried bone preparations are then exposed to low-dose gamma radiation for sterilization and vacuum-sealed in sterile containers. This process is followed for every bone donation to assure the allograft bone is safe to use (Mellonig 95). The likelihood of viral transmission to recipients by donor bone is 1 in 2.8 billion (Mellonig 93, Mellonig 95).

3. A xenograft is defined as "A heterograft" (AAP Glossary of Terms) meaning it is a graft material obtained from a different species. The most common xenografts are bovine and porcine in origin.

4. An alloplast is defined as "A synthetic graft or inert foreign body implanted into tissue" (AAP Glossary of Terms). Examples of alloplast grafts include hydroxyapatite crystals and bioactive glass. No histologic evidence has been published that shows true periodontal regeneration

using alloplasts or xenografts. This may be because alloplastic materials are not resorbed and turned into new bone as are autografts and allografts. Alloplasts heal by a process of fibrous encapsulation and will never be turned over into new bone. Xenografts will be resorbed and turned into new bone, but the process is very lengthy and not amenable to regenerative therapy.

Demineralized freeze-dried bone allograft (DBFBA)-Bone morphogenetic proteins (BMP)

Of the available graft materials, allografts are the choice material for periodontal regeneration (Mellonig 81) and not just because they negate the need for a donor surgical site. Although demineralized or mineralized bone allograft can be used, DFDBA is preferred because de-mineralization unmasks BMP that induce greater osteo-inductivity; the ability to induce stem cells to become bone-forming cells. DFDBA has been shown to be even more osteo-inductive than autograft bone (Mellonig 81). BMP are differentiation factors that cause other cells to differentiate into bone forming cells. BMP are thought to play a role in periodontal regeneration (Ripamonte 94). When higher amounts of BMPs are present, an increase in the amount of intramembranous bone formation results (Wozney 02). This is ideal for jaw bones since they develop via intramembranous bone formation. In periodontal regeneration therapy, DFDBA with its BMPs helps form new bone faster and fills in the diseased areas with healthy bone more completely than FDBA (Mellonig 81).

Numerous studies have evaluated periodontal regeneration surgery in intrabony defects using DFDBA with a membrane, DFDBA alone, and a membrane alone (Cortellini 93, Cortellini 96, Trejo 00, Guillemin 93). A membrane is “A thin sheet-like usually nonautologous material used in various periodontal regenerative procedures” (AAP Glossary of Terms). A membrane serves four purposes in periodontal regeneration: 1) space maintenance (keep space for new tissues to grow), 2) blood clot stabilization, 3) wound stabilization, and 4) epithelial exclusion (keep the epithelium away from the side of the tooth so the bone, PDL cells and cementum can regenerate the defect). In a series of studies published by Bowers and colleagues. in 1989, histologic evidence of true periodontal regeneration (new bone, cementum, and PDL) was observed using DFDBA without a membrane, and with DFDBA and a free gingival graft acting as a membrane. In a study by Cortellini and colleagues. in 1993, periodontal regeneration of intrabony defects was accomplished with the use of a membrane alone with 40-95% bone fill was achieved depending on the size and shape of the bony defect. Additional studies utilizing DFDBA plus a membrane found greater bone fill in the defects using the combination therapy; DFDBA

plus a membrane (Guilleman 93, Schallhorn 88). The benefit of bone grafting is more evident in deeper bone defects (where the intrabony depth upon surgical exposure is ≥ 4mm) than in shallower defects (Laurell 98).

Membranes

Once the bone graft has been placed in the bony defect around the tooth, the next step in periodontal regeneration involves the placement of a membrane around the tooth and under the replaced tissue flap. In addition to excluding gingival epithelium from the defect so that lost PDL, cementum and alveolar bone have time to regenerate, membranes also help contain the bone graft material and stabilize the post-surgical blood clot. Both resorbable and non-resorbable membranes are available for periodontal regenerative surgery. Resorbable membranes are made from a synthetic material or are processed from bovine or porcine collagen. They provide stability and function up to 8 weeks before the healing process dissolves them. Non-resorbable membranes are synthesized from an expanded polytetrafluoroethylene material, better known as Gore-tex. These require a second surgery to remove the membrane after healing. Since no difference in bone fill outcomes have been shown between resorbable and non-resorbable membranes (Garrett 97, Cortellini 96), and a second surgery is needed to remove non-resorbable membranes, resorbable membranes are preferred for periodontal regenerative procedures.

**CLINICAL AND RADIOGRAPHIC ASSESSMENT**

Following regenerative surgery, the only way to confirm whether new bone, cementum and PDL have formed in the defect is through histological examination of the site. However, histology requires extraction of a tooth and its surrounding supporting tissues which is not a feasible choice. Therefore, clinical parameters and radiographic evaluation are used to determine clinical success of the regenerative procedures (Machtei 97).

Clinical parameters used to evaluate regenerative therapy include:

- probing depth - distance from the soft tissue gingiva or alveolar mucosa margin to the bottom of periodontal pocket using a periodontal probe
- clinical attachment level- distance from the cemento- enamel junction to the soft tissue base of the periodontal pocket using a periodontal probe
- recession - distance from the cementoenamel junction to the gingival margin when root surface

has become exposed

- bleeding on probing- an indication of gingival inflammation that occurs after periodontal probing, diseased sites bleed, healthy site do not bleed

Radiographic assessments using standardized digitized images include:

- Change in bone height from bottom of the intrabony defect (base of the bony pocket) to cement-enamel junction on pre and postsurgical digital radiographs.
- Measurement from the CEJ to the alveolar (bone) crest will be collected as a constant to determine the proportion of bony defect fill.
- Measurement from the CEJ to the apex (end of the tooth root) of the tooth will be made to assist in establishing radiographic quality control and in determining the percentage of bone fill.

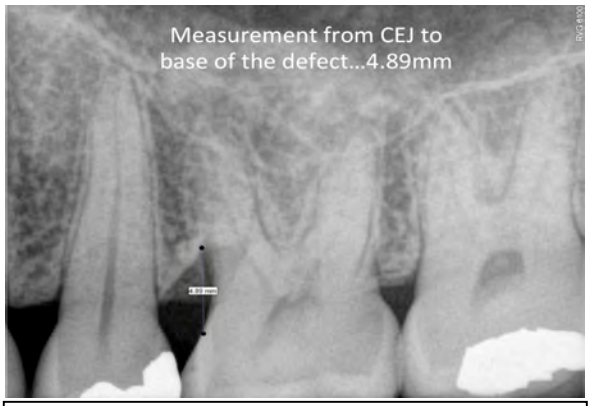



Fig 5: Example of radiographic measurement from CEJ to base of intrabony defect

  - At NPDS, all dental radiographs are digital and stored on the NNMC-DDILOCAL database, and XrayVision is the software used for viewing, and assessing the images. This software has measuring tools that can assess changes in bone levels before and after surgery.

* If NPDS procures subtraction radiography software then change in bone volume of intrabony defects between baseline and post-operatively at 6 months and 12 months (Machtei 97) will be assessed. This assessment would use the existing pre and postsurgical digitized radiographs that each participant receives as part of the protocol. Subtraction radiography can compare baseline bone defect volume to the post-operative defect bone fill (Hausmann 90, Hausmann 00) using the standardized digital radiographs. It is defined as a "digital method of attenuating the image representing unchanged structures from the compared radiographic images of the same anatomic image, thereby making changes easier to detect" (AAP Glossary of Terms). Using digital radiographs "a radiographic image subtraction method in which the pre- and post-procedure radiographic images are subtracted from each other within the computer memory

and the resulting subtracted image is displayed on the monitor." (AAP Glossary of Terms). Subtraction radiography determines changes bone volume by assessing differences in mineralization before and after surgery. The technique can differentiate changes as small as 5% (Guimareaes 10). Volumetric assessment of bone grafting effect may provide useful data about the benefit of bone grafting that linear measurement of clinical and radiographic parameters cannot provide.


Fig 6: Hydrated DFDBA

**SUMMARY**

In summary, particulate DFDBA with root biomodification with tetracycline or other acids like citric acid or ETDA has been a standard of care for decades in periodontal regenerative surgery (Bowers 89, Reynolds 96). Some clinicians use membranes and some do not. One of the challenges of DFDBA is its particulate nature, especially in large defects with minimal walls to hold the graft material. Since defects with less than 3 walls make keeping particulate DFDBA in place more difficult, membranes are often used to aid bone graft retention while the blood clot is organizing.

Fig 7: Accell – putty-like consistency

Accell connexus is a new bone allograft material approved by the FDA for use in periodontal regeneration. It is demineralized freeze-dried bone allograft mixed in a proprietary reverse phase medium (Keystone Dental) which makes it appear and feel like grits. This medium allows the material to act like a putty material and enables the practitioner to mold and shape the allograft in the defect site. Clinical observations suggest that Accell connexus stays in place better than traditional particulate DFDBA.

Accell connexus is also unique because during its processing, an additional step allows Accell connexus to have 5-7 times more BMP than traditional demineralized bone allograft. This additional step involves splitting a large sample of freeze dried bone allograft into two parts. One part is demineralized as discussed in the background to unmask its BMP, while the other part has its BMP thoroughly extracted via

demineralization, collected, and then the extracted BMP is transferred to other ½ sample. This process results in the increased concentration of BMP compared to traditional DFDBA. Final sterilization occurs after the bone allograft has received the extra BMP. (Appendix A2 illustrates the production of Accell).

Numerous articles have been published on periodontal regeneration of intrabony defects with and without DFDBA, with and without membranes, and with and without biologic mediators (bone morphogenetic proteins, growth factors, recombinant proteins) (Laurell 98, Bender 05, Blumenthal 90, Boyan 06, Caton 97, Murphy 03, Nevins 03, Reynolds 03). However, no articles have been published that compare Accell connexus to the gold standard bone allograft material for periodontal regeneration, DFDBA.

Our objective with this study is to assess whether Accell connexus, which is DFDBA with additional bone morphogenetic proteins produced in a putty like matrix, will be as efficacious as or more efficacious than DFDBA in periodontal regenerative therapy.

## MATERIALS AND METHODS

Thirty subjects diagnosed with severe periodontitis will be enrolled in the study. (Please see Appendix A1 for a flow diagram of the proposed study.) The findings of their comprehensive periodontal evaluation such as probing depths (PD), clinical attachment levels (CAL), and recession will have been recorded on the Navy Periodontal Chart Form - NAVMED 6660/2 (appendix B2) by the subject's provider. If the patient meets the inclusion criteria for the study, they will be offered the opportunity to participate. The methodology for this study is listed below in sequential order.

The inclusion and exclusion criteria for this study included the following:

**Inclusion Criteria**

a. Patient aged ≥18 years old
b. Patient will be remaining in the Capital region for at least 12 months following the surgical procedure for follow up appointments
c. Diagnosis of generalized or localized severe periodontitis
d. Radiographic evidence of a vertical intrabony defect at one or more sites with a probing depth ≥ 6 mm
    1. If the patient present with more than one defect site meeting inclusion criteria, the site with the deepest probing depth will be used in the study

**Exclusion Criteria**

a. Patient under the age of 18
b. Patient will be moving from the Capital region area prior to 12 months following the surgical treatment
c. Furcation involvement in combination with the intrabony defect determined pre-surgically
d. Patients with restorations extending beyond the cementoenamel junction at the intrabony defect site
e. Patients with an indiscernible cementoenamel junction either clinically or radiographically

f. Patients with periapical pathology, unrestored caries, defective restorations, root resorption, or vertical root fracture
g. Patients requiring restorative dental care (fillings and crown and bridge work) that cannot be completed prior to fabrication of the customized stent
h. Female patients who are pregnant or nursing
i. Patients who currently smoke tobacco or use tobacco products. Former smokers will be excluded if they quit smoking < 6 months prior to selection in the study.
j. Patients with clinically significant systemic diseases, which may affect healing (e.g. uncontrolled diabetes).
k. Patients allergic to chlorhexidine gluconate (Peridex).
l. Patients allergic to tetracycline
m. Patients with poor oral hygiene unsuitable for periodontal surgery
n. Patients who cannot or will not sign the informed consent form
o. Patients receiving immunosuppressive therapy such as chemotherapy and systemic corticosteroids not to include inhaled or topical steroids
p. Patients with severe endocrine-induced bone diseases (e.g. hyper-thyroidism, altered parathyroid function)
q. Patients with bleeding complications (e.g. hemophilia)
r. Patients on warfarin therapy
s. Patient with a history of osteoporosis or taking bisphosphonate medications
t. Patients with a history of radiation therapy in the head and neck area
u. Patients whose teeth with intrabony defect have mobility classified as Miller class 2 or greater

**Initial Sequence:**

2. Patient is referred for a comprehensive periodontal evaluation.
3. Initial therapy in the form of scaling and root planing is accomplished by a registered hygienist, periodontist, or periodontal resident.
4. 4 to 6 weeks following initial periodontal therapy, the patient's initial therapy is re-

evaluated to assess healing and oral hygiene.

5. A 2nd full periodontal charting will be completed at re-evaluation; including probing depth measurements, clinical attachment level measurements, bleeding on probing, and plaque scores for each tooth.
6. Based on the re-evaluation a treatment plan will be developed for each patient. Typical treatment plans are:
    1. Maintenance therapy. No surgical treatment required, patient is not a candidate for the study.
    2. Surgical treatment required, but regenerative therapy is not indicated, the patient is not a candidate for the study.
    3. Intrabony vertical defect is present, but site has furcation involvement. The patient is not a candidate for the study.
    4. Intrabony vertical defect is present and regenerative therapy is the treatment of choice.
        - Patient will be asked if he/she would like to participate in the study and will then be provided a one page brief about the study
            i. If the patient consents to be in the study, the therapy will continue as stated below
            ii. If the patient does not consent to be in the study, surgical therapy will continue as planned by the patient's surgeon.

**Following Consent:**

1. Maxillary and mandibular impressions using an irreversible hydrocolloid material (alginate) will be made using stock impression trays; sized small, medium, or large depending on the size of the subject's mouth. The impressions will be poured with dental stone. The stone models of the subject's jaws will be used to fabricate a customized plastic stent to allow standardized measurements of the surgical site.
    a. Plastic stent fabrication:
        i. A plastic stent for making probing depth measurements will be fabricated utilizing the methods described by Isador 84 and Deas 04.


Fig 8: Example of the customized stent used for data collection

ii. A 2 mm thick co-polyester plastic dental splint material (biocryl material) will be adapted to the stone model of the subject's arch utilizing a BioStar matrix machine.

iii. The stent will be trimmed to end just above the height of contour of the crowns of the teeth in order to visualize the gingiva.

iv. A fissure bur (1169 bur) will be used to cut grooves in the interproximal areas and along the buccal and lingual aspects of the teeth being investigated. These grooves accommodate the periodontal probe and allow the investigator to probe the same location and with the same angulation at pre or post surgical visits.

v. Following use the stent will be cleaned and disinfected with Dispatch spray and stored in a ziplock plastic bag labeled with the subject's study number. The bag will be locked in a secured drawer maintained by the primary examiner; and then retrieved for measurements at 6 and 12 months.

2. A customized bite-plate registration using the paralleling radiographic technique will be fabricated for each patient to standardize radiographs at baseline and 6 and 12 months after surgery.

i. A Rinn film holder used for the paralleling technique will be selected based upon the size of the sensor used for the digiradiographs:

1. Size 1 for individuals with smaller mouths
2. Size 2 for individuals with larger mouths.

ii. Blu-mousse, a bite registration material, will be applied to each side of the film holder where the teeth contact the holder>

1. Subjects bite into blu-mousse until the material hardens (approximately 45 seconds).
2. The film holder is removed from the mouth.
3. The film holder is reinserted into

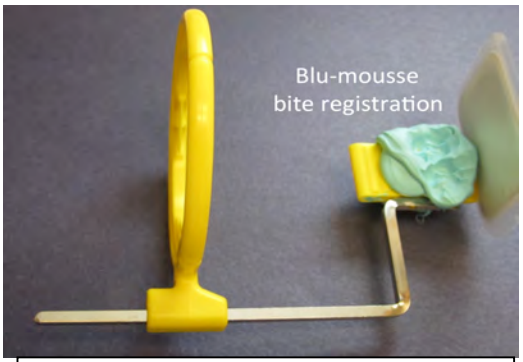



Fig 9. Example of customized film holder using Blu Mousse bite registration material

the mouth and the subject bites down to confirm that the bite is reproducible.

iii. *Following use, the film holder will be cleaned and disinfected with Dispatch spray and stored in a ziplock plastic bag labeled with the subject's study number and locked in a secured drawer maintained by the primary examiner; and then retrieved for postoperative radiographs at 6 and 12 months.

3. Prior to surgery, clinical parameters will be measured using the customized plastic stent* and a UNC-15 periodontal probe. All clinical measurements will be made by a blinded study investigator.
    a. Probing depth: measured in millimeters from the gingival margin to the base of the pocket/
    b. Clinical attachment level: Measured from the cementoenamel junction of each tooth to the soft tissue base of the pocket.
    c. Recession: Measured from the cementoenamel junction of the tooth to the gingival margin.
    d. Bleeding on probing: 30 seconds following measurements of the probing depth and clinical attachment level, the area will be re-examined.
        i. The presence or absence of bleeding will be recorded on the data collection sheet.
    e. Plaque score: The presence or absence of plaque at the defect site will be recorded on the data collection sheet.
4. Prior to surgery, a standardized digital periapical radiograph will be made using the customized bite-plate and the paralleling technique.
    a. All radiographs made at NPDS are stored on the NNMC-DDILOCAL radiographic database and are viewed using the software XrayVision DCV. This database is secured. The database can only be accessed by authorized CAC users. Radiographs are identified by the patient's full name, social security number and date image was made.

**Randomization Procedure:**

1. A computer program will randomly sequence each subject's study enrollment numbers (1-30) as in the example below.

a. A random sequence table will be generated by the research coordinator following IRB approval in order to maintain blinding of investigators.

Random Sequence Generator:

http://www.random.org/sequences/?min=1&max=30&col=2&format=html&rnd=new

| Group A. DFDBA | Group B. Accell |
|---|---|
| 2 | 4 |
| 25 | 15 |
| 30 | 28 |
| 14 | 10 |
| 24 | 6 |
| 1 | 21 |
| 11 | 7 |
| 5 | 26 |
| 19 | 17 |
| 13 | 9 |
| 16 | 12 |
| 22 | 23 |
| 3 | 20 |
| 29 | 27 |
| 18 | 8 |

Timestamp: 2012-05-31 18:54:03 UTC

2. Thirty envelopes marked 1 -30 will contain either a card stating DFDBA or Accell. Thereafter, the random sequence table will be placed in a sealed envelope that will not be opened until all data has been collected. Sealed envelopes (1-30) will be stored by the principal investigator in a locked drawer.
3. When each participant goes to surgery the investigator will provide the surgical team the envelope corresponding to that subject's enrollment number. The surgical team will open the envelope and remove a card which will state which bone graft material to place following debridement and categorization of the defect.

a. A surgical team member will check on the card whether the intrabony defect was ideal for bone grafting, ≥ 4mm in depth or less than ideal, < 4mm in depth, and if a furcation was evident that was not detected pre-surgically.
    i. Please note that surgical findings sometimes differ from pre-surgical estimates of the surgical site.

    One of the cards below will be sealed in each envelope (1-30).

| **Accell** | |
|---|---|
| __ defect≥4mm | __ defect<4mm |
| | __ furcation evident |

| **DFDBA** | |
|---|---|
| __ defect≥4mm | __ defect<4mm |
| | __ furcation evident |

4. The completed card will be returned to the numbered envelope and resealed. The study investigator will collect the sealed envelope and place it in the data collection folder for each study participant that is stored by the principal investigator in a locked drawer.
5. The study investigators who make the postsurgical clinical and radiographic assessments at 6 and 12 months will be blinded to which bone graft material a given participant received.

**Surgical Procedure:**

Females of child bearing age will be asked to complete a HCG urinalysis prior to the surgical procedure. If the results of the HCG test are positive, the subject will be exited from the study.

Prior to surgical procedure, in line with standard procedure at the Periodontics Department participants will be offered the option of having the surgery performed using: 1. only local anesthesia, or 2. a combination of oral anxiolysis with Triazolam and local anesthesia, or 3. a combination of IV moderate sedation with Versed and Fentanyl and local anesthesia. The use of sedation will not affect the surgical procedure.

1. The surgical provider will be either a board certified staff periodontist or a 2nd or 3rd year periodontal resident. All surgical providers will be briefed in the protocol. All surgeries will follow the same steps listed below.
    a. Surgical set-up is standardized for all surgeries done at the Naval Postgraduate Dental School Periodontics Department.

b. Both the experimental (Accell) and control (DFDBA) materials will be available to the surgeon. The bone graft material used will be determined when the sealed envelope is opened by a surgical team member after the defect has been debrided and characterized.

c. Surgical Procedure Steps:

    i. Placement of normal saline IV
        1. Administration of 8mg Dexamethasone IV

    ii. Administration of oral anxiolysis or IV moderate sedation if patient desired and indicated

    iii. Administration of topical and local anesthetic with any combination of 2% Lidocaine with 1:100K epinephrine, 4% Articane with 1:100K epinephrine, and 0.5% Marcaine with 1:200K epinephrine

    iv. Sulcular incisions and full thickness reflection of the surgical flap

    v. Debridement of the surgical site/defect to remove granulation tissue and calculus using hand instruments and cavitron ultrasonic instrument

    vi. Characterization of the defect by a study investigator
        1. Number of defect walls present: 1-, 2-, 3-walled defect or combination defect
        2. Depth of defect from CEJ to the base of the bony pocket
        3. Depth of defect from the alveolar crest to the base of the bony pocket
        4. Mesial-distal defect width: Measured in the mesial-distal direction from the tooth to the mesial or distal margin of the defect
        5. Buccal-lingual defect depth: Measured in the buccal-lingual direction from the buccal margin of the defect to the lingual margin of the defect
        6. Following defect characterization, the investigator provides the surgical team with the sealed envelope to determine which bone graft material, Accell or DFDBA, the participant was randomized to receive, and the investigator leaves the surgical suite.

    vii. The graft material will be prepared as defined by the manufacturing instructions:
        1. Hydration of the graft material with sterile saline - DFDBA
        2. Graft material dispensed from syringe - Accell.

    viii. The root surface of the tooth bordering the defect site will be treated with a 24% EDTA gel f for 4 minutes. The site will then be washed with sterile saline for 1 minute.

ix. Osteoplasty (reshaping unsupported the alveolar bone) will be performed as needed
x. Intramarrow penetration of the bone within the defect using a ¼ surgical round bur to induce bleeding in the defect site
xi. Graft material (determined from the sealed envelope) placed into the defect up to the level of the alveolar crest
xii. Bio-Gide membrane trimmed and positioned to cover grafted defect
xiii. Primary flap closure achieved using a non-resorbable monofilament suture (ie. Gore-tex)
xiv. Gauze pressure will be held on the site for 5 minutes to achieve hemostasis and reduce the size of the fibrin clot formed.
xv. Periodontal dressing may be placed over the surgical site.
xvi. Surgical team checks appropriate findings on randomization card, reseals card in envelope, and envelope collected by investigator.
    1. Envelopes will not be re-opened until after data analysis

**Post-operative Care:**

1. All participants receive the following post-operative regimen:
    a. Pain medication consisting of any of the following alone or in combination:
        i. Ibuprofen 800 mg , Take 1 tab PO q6-8h for moderate pain
        ii. Hydrocodone/Acetaminophen 5/325 mg, Take 1-2 tab PO q6h prn severe/breakthrough pain
        iii. Oxycodone/Acetaminophen 5/325mg, Take 1-2 tab PO q6h prn severe/breakthrough pain
    b. Pain medication for patients who cannot take NSAIDS will be prescribed any of the following alone or in combinations:
        i. Acetaminophen 325 mg, Take 1-2 tabs PO q4h for moderate pain
        ii. Oxycodone 5mg, Take 1 tab PO q4h prn severe/breakthrough pain
    c. Antibiotics consisting of either of the following:
        i. Amoxicilin 500mg, Take 1 tab PO q8h for 10 days
        ii. Clindamycin 300 mg, Take 1 tab PO q8h for 10 days
    d. 0. 12% Chlorhexidine, 1 bottle, Rinse and spit bid with 1 TBSP as directed on the bottle

2. All patients are provided with the standard post-operative instructions (See appendix B3 for an example of the standard postoperative care instruction form).
3. Patients are recalled at 1 week to assess post-operative healing and remove plaque/deposits on the surgical site.
4. Patients recalled at 2 weeks post operative to assess healing, remove plaque, and remove sutures at the surgical site.
5. Patients recalled at weeks 4, 6, 8, 12, and 16 to assess healing, remove plaque, and reinforce oral hygiene.
6. Patients recalled at 6 months following the surgical procedure to assess healing, remove plaque, and reinforce oral hygiene.
    a. A study investigator blinded to the graft material used will evaluate the periodontal parameters using the customized stent and take a periapical radiograph using the customized bite-plate and paralleling technique.
        i. Same methods as in pre-surgical evaluation
        ii. If the customized stent is not stable on the patient's teeth at the follow-up appointment, the clinical data will not be used in the analysis. The radiographic data will still be collected.
7. Patients recalled at 9 months for periodontal maintenance therapy
8. Patient recalled at 12 months following the surgical procedure to assess healing, remove plaque, and reinforce oral hygiene.
    a. A study investigator blinded to the graft material used (other than the surgeon or the staff member on the surgical case) will evaluate the periodontal parameters using the customized stent and take a periapical radiograph using the customized bite-plate and paralleling technique.
        i. Same methods as in pre-surgical evaluation
        ii. If the customized stent is not stable on the patient's teeth at the follow-up appointment, the clinical data will not be used in the analysis. The radiographic data will still be collected.
9. Patient will be exited from the study and followed by their primary provider for periodontal maintenance therapy.

**Analysis of Data:**

1. Periodontal parameters assessed at 6 months and 12 months will be compared to the baseline measurements to determine change in clinical attachment level and probing depth.
    a. A comprehensive periodontal charting (probing depths, attachment levels, bleeding on probing, plaque score) for all teeth present in the mouth will be done at the 12 months visit as well.
2. Two reviewers, board certified periodontist(s) and/or a board certified oral radiologist, blinded to which bone graft material subjects received will access the NNMC-DDILOCAL database, and use the XrayVision software used for viewing to measure bone levels before surgery and at 6 months and 12 months.
    a. Radiographic analysis will be completed following data collection
    b. To access the radiographs, the examiners will be provided with a sub-master list containing the study number, name, and last four of the social security number.
    c. The examiners will access the patient's radiographic record on the NNMC-DDILOCAL database using the patient's name and last four.
    d. The standardized radiographs taken at baseline, 6 months following surgery and 12 months following surgery will be obtained.
    e. Using the digital radiograph software, measurements will be made and recorded on the data collection sheet for radiographs.
    f. The sub-master list will be destroyed following all measurements.
3. If subtraction radiography becomes available at NPDS, the same radiographs will be used to assess changes in bone volume from baseline to 6 and 12 months postoperatively.
4. Statistical analysis will assess pre and post test differences.

## RESULTS

At the time of writing this thesis, there are no results to report on. Patients are currently being enrolled in the study. Due to the need for six month and one year follow-up on all cases, data will not be available until at least July 2013. At present, one surgical procedure has been completed and another is scheduled to occur in March/April 2013. We continue to actively pursue patient enrollment with the goal of having all patients enrolled by the summer of 2013.

Data will be collected and analyzed over the next year by the associate investigators. Once all patients have completed the twelve month follow-up appointment, all data will be analyzed for significance. The data that will be collected includes the following:

Clinical Measurements:

The clinical measurements will be made and recorded on the data collection sheet (Appendix B2) prior to surgery, 6 months following surgery, and 1 year following surgery. A customized stent made from lab plastic material and a sterile UNC-15 periodontal probe, the standard probe used by the Periodontics Department, will be used to assess all clinical measurements recorded on the periodontal chart and data collection sheet. Probing depth and clinical attachment level are measured to the nearest mm.
Note: Separate data collection sheets are used for clinical measurements before surgery, at 6 months and at 12 months to blind the investigator to previous measurements as well as to bone graft material used.

Defect Characterization:

The defect characterization measurements will be made and recorded on the data collection sheet following debridement of the intrabony defect. A sterile UNC-15 periodontal probe, the standard probe used by the Periodontics Department, will be used characterize the intrabony defect during surgery and measurements will be recorded on the data collection sheet.

Radiographic Data:

Following fabrication of a customized bite-plate made from blu-mousse bite registration material and a paralleling device, a standard periapical radiograph will be taken using the digital radiography program used by the Periodontics Department prior to surgery, 6 months following surgery, and 1 year following surgery. Radiographic data will be assessed after data collection is complete.

The primary outcome variable is the change in the clinical attachment levels from baseline to 6 months and 12 months following surgery.

The secondary outcome variables include the changes of probing depths and changes in linear measurements of bone fill on radiographs from baseline to 6 months and 12 months post surgery. If subtraction radiography becomes available at NPDS, then the percentage change of radiographic bone volume from baseline to 6 months and 12 months post operatively will also be assessed.

The null hypothesis for this study is that there will no difference between the Accell and DFDBA in terms of clinical outcomes and radiographic bone fill.

**Statistical Analysis:**

This study compares the pre and post surgical changes in clinical attachment levels and linear measurement of bone fill on radiographs in the two treatment groups: group A, using DFDBA which is the current gold standard, and group B, Accell which could become the gold standard. Data for pre and post surgical changes will be analyzed using the Mann-Whitney U test.

There have been multiple studies examining the efficacy of DFDBA and comparing this product to other products. There have currently been no studies done comparing DFDBA to Accell. The sample size for this study will be 30 subjects (15 subjects for each arm of the study). The sample size is based upon a study done by Hoidal and colleagues, 2008. In the study by Hoidal, "A sample size of at least 28 patients with at least one defect (14 DFDBA and 14 DFDBA/EMD) was established to detect a clinically significant mean difference of at least one standard deviation at the 0.05 level with power of 80%. This was determined using a population standard deviation of bone fill ≤1.0 mm, allowing for the detection of a clinically significant mean bone fill difference between treatment groups ≥1.0 mm." The 2 extra subjects have been requested in case some subjects exit the study.

## DISCUSSION/CONCLUSION

The purpose of this blinded randomized parallel-arm controlled clinical trial was to determine if Accell connexus, a demineralized freeze-dried bone allograft product that contains 5-7 times the amount of bone morphogenetic proteins as regular demineralized freeze-dried bone allograft (DFDBA) provides superior periodontal regeneration (formation of new bone, cementum, and connective tissue around teeth) than regular demineralized freeze-dried bone allograft. At present since no data has been analyzed, we cannot draw any conclusions or make any statements regarding the efficacy of the material.

Particulate DFDBA with root biomodification with tetracycline or other acids like citric acid or ETDA have been a standard of care for decades in periodontal regenerative surgery (Bowers 89, Reynolds 96). Some clinicians use membranes and some do not. One of the challenges of DFDBA is its particulate nature, especially in large defects with minimal walls to hold the graft material. Since defects with less than 3 walls make keeping particulate DFDBA in place more difficult, membranes are often used to aid bone graft retention while the blood clot is organizing.

Numerous articles have been published on periodontal regeneration of intrabony defects with and without DFDBA, with and without membranes, and with and without biologic mediators (bone morphogenetic proteins, growth factors, recombinant proteins) (Laurell 98, Bender 05, Blumenthal 90, Boyan 06, Caton 97, Murphy 03, Nevins 03, Reynolds 03). However, no articles have been published that compare Accell connexus to the gold standard bone allograft material for periodontal regeneration, DFDBA.

Accell connexus is a new bone allograft material approved by the FDA for use in periodontal regeneration. It is demineralized freeze-dried bone allograft mixed in a proprietary reverse phase medium (Keystone Dental) which makes it appear and feel like grits. This medium allows the material to act like a putty material and enables the practitioner to mold and shape the allograft in the defect site. Clinical observations suggest that Accell connexus stays in place better than traditional particulate DFDBA.

Of the available graft materials, allografts are the choice material for periodontal regeneration (Mellonig

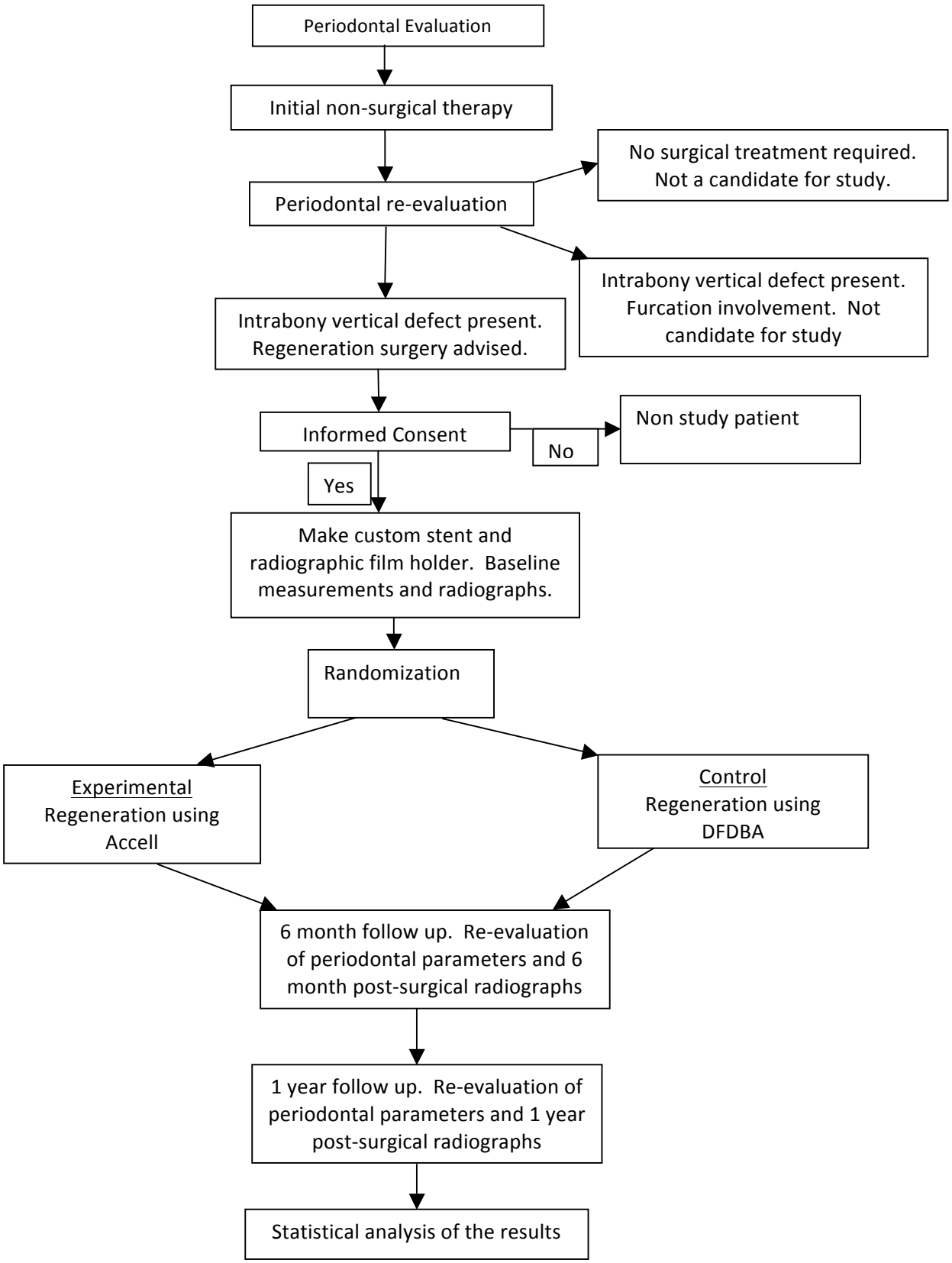
81) and not just because they negate the need for a donor surgical site. Although demineralized or mineralized bone allograft can be used, DFDBA is preferred because de-mineralization unmasks BMP that induce greater osteo-inductivity; the ability to induce stem cells to become bone-forming cells. DFDBA has been shown to be even more osteo-inductive than autograft bone (Mellonig 81). BMP are differentiation factors that cause other cells to differentiate into bone forming cells. BMP are thought to play a role in periodontal regeneration (Ripamonte 94). When higher amounts of BMPs are present, an increase in the amount of intramembranous bone formation results (Wozney 02). This is ideal for jaw bones since they develop via intramembranous bone formation. In periodontal regeneration therapy, DFDBA with its BMPs helps form new bone faster and fills in the diseased areas with healthy bone more completely than FDBA (Mellonig 81).

Accell connexus is also unique because during its processing, an additional step allows Accell connexus to have 5-7 times more BMP than traditional demineralized bone allograft. This additional step involves splitting a large sample of freeze dried bone allograft into two parts. One part is demineralized as discussed in the background to unmask its BMP, while the other part has its BMP thoroughly extracted via demineralization, collected, and then the extracted BMP is transferred to other ½ sample. This process results in the increased concentration of BMP compared to traditional DFDBA. Final sterilization occurs after the bone allograft has received the extra BMP.

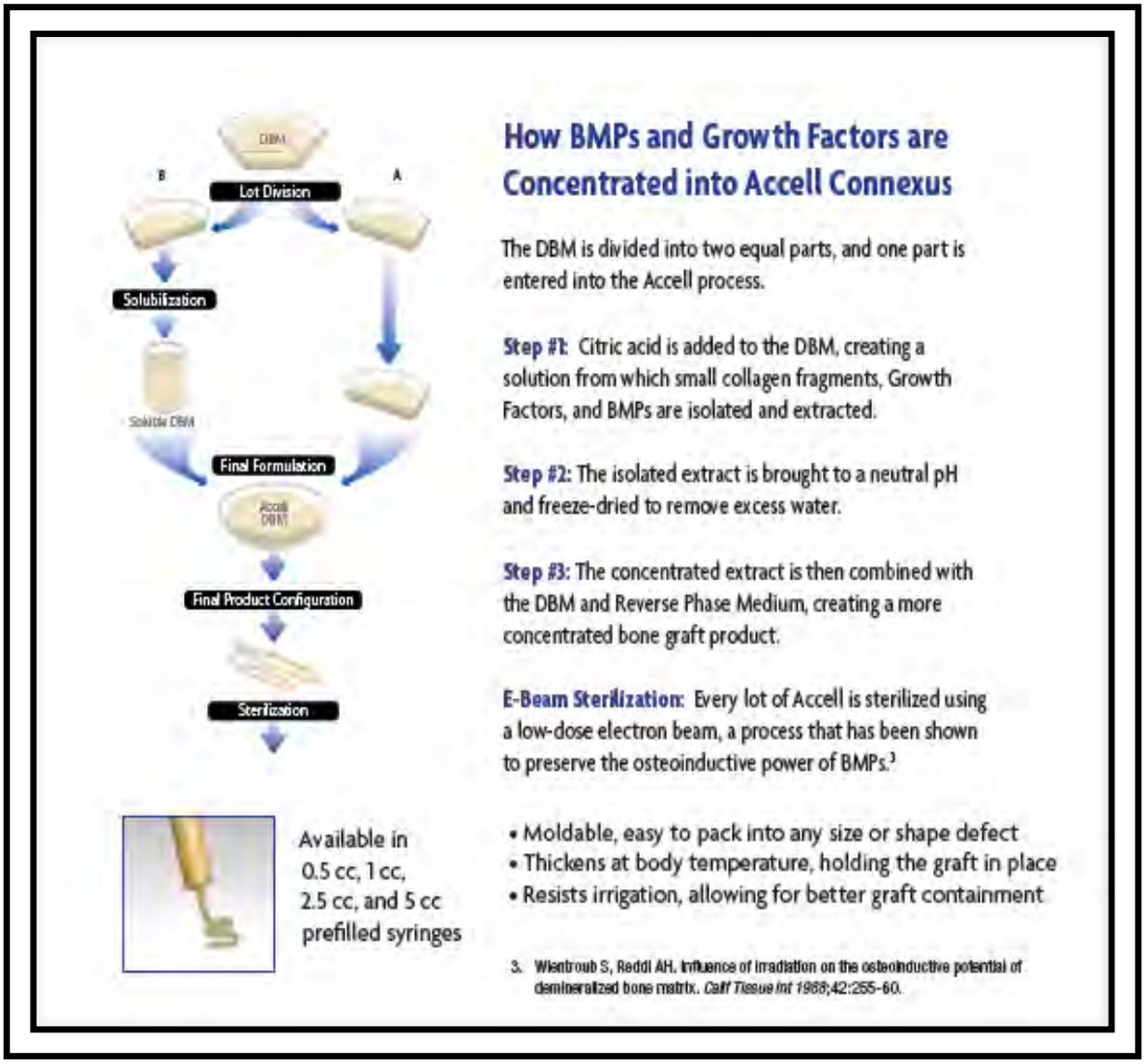
Clinical experience with the product Accell connexus has left us with a positive feeling towards the product. The ease of use and moldability of the graft material allows it to be placed into all types of defects with ease. Clinical results have been favorable so far in terms of clinical measurements and radiographic data. Although we cannot draw any conclusions at this time, based upon clinical experience we feel as though Accell connexus will outperform DFDBA in terms of improved clinical attachment levels and improved radiographic bone fill at both six and twelve months post surgery.

**APPENDIX A**

## Appendix A1: FLOW DIAGRAM OF STUDY DESIGN



**Appendix A2: Accell Brochure provided by Keystone Dental**




Permission to use Accell connexus graphic provided by the company, Keystone Dental. This image is published on all brochures relating to the product Accell.

## APPENDIX B

### Appendix B1: Data Collection Sheets

A master list will associate participant's name with the last 4 numbers of their social security number, telephone number and email address. Each of the following boxes (data collection sheets) will be printed on separate pieces of paper.

**Periodontal Regeneration of 1-, 2-, 3-Walled Intrabony Defects Using Accell Connexus vs DFDBA: A Randomized Parallel Arm Clinical Trial**

Date _____
Subject ID # _____
Gender _____
Age _____

| **Clinical Measurements:** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tooth #: | | | | | | | | | | | | |
| | Probing Depth (mm) | | Clinical Attachment Level (mm) | | Recession (mm) | | Plaque Score (+/-) | | BOP (+/-) | | | |
| | M/D-B | M/D-L | M/D-B | M/D-L | M/D-B | M/D-L | M/D-B | M/D-L | M/D-B | M/D-L | | |
| **Baseline** | | | | | | | | | | | | |

**Periodontal Regeneration of 1-, 2-, 3-Walled Intrabony Defects Using Accell Connexus vs DFDBA: A Randomized Parallel Arm Clinical Trial**

Date _____
Subject ID # _____
Gender _____
Age _____

| **Clinical Measurements:** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tooth #: | | | | | | | | | | | | |
| | Probing Depth (mm) | | Clinical Attachment Level (mm) | | Recession (mm) | | Plaque Score (+/-) | | BOP (+/-) | | | |
| | M/D-B | M/D-L | M/D-B | M/D-L | M/D-B | M/D-L | M/D-B | M/D-L | M/D-B | M/D-L | | |
| **6 months** | | | | | | | | | | | | |

**Periodontal Regeneration of 1-, 2-, 3-Walled Intrabony Defects Using Accell Connexus vs DFDBA: A Randomized Parallel Arm Clinical Trial**

Date _____
Subject ID # _____
Gender _____
Age _____

| **Clinical Measurements:** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Tooth #: | | | | | | | | | | |
| | Probing Depth (mm) | | Clinical Attachment Level (mm) | | Recession (mm) | | Plaque Score (+/-) | | BOP (+/-) | |
| | M/D-B | M/D-L | M/D-B | M/D-L | M/D-B | M/D-L | M/D-B | M/D-L | M/D-B | M/D-L |
| **12 months** | | | | | | | | | | |

**Periodontal Regeneration of 1-, 2-, 3-Walled Intrabony Defects Using Accell Connexus vs DFDBA: A Randomized Parallel Arm Clinical Trial**

Date _____
Subject ID # _____
Gender _____
Age _____

| **Surgical Measurements (Characterization of Defect):** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Tooth #: | | | | | | | | | | |
| | Defect Classification (1,2,3-walled or combination) | | CEJ-Base of Defect (mm) | | Depth of Defect: Alveolar Crest-Base of Defect (mm) | | Width of Defect: M-D Width (mm) | | Width of Defect: B-L Width (mm) | |
| | M/D-B | M/D-L | M/D-B | M/D-L | M/D-B | M/D-L | M/D-B | M/D-L | M/D-B | M/D-L |
| At Surgery | | | | | | | | | | |

**Periodontal Regeneration of 1-, 2-, 3-Walled Intrabony Defects Using Accell Connexus vs DFDBA: A Randomized Parallel Arm Clinical Trial**

Date _____
Subject ID # _____
Gender _____
Age _____

| **Radiographic Measurements** | | | | | | |
|---|---|---|---|---|---|---|
| Tooth #: | | | | | | |
| | CEJ-Base of Defect (mm) | | CEJ-Apex of Tooth (mm) | | CEJ-Alveolar Crest (mm) | |
| | M/D | M/D | M/D | M/D | M/D | M/D |
| Baseline | | | | | | |
| 6 months | | | | | | |
| 12 months | | | | | | |

**_____**

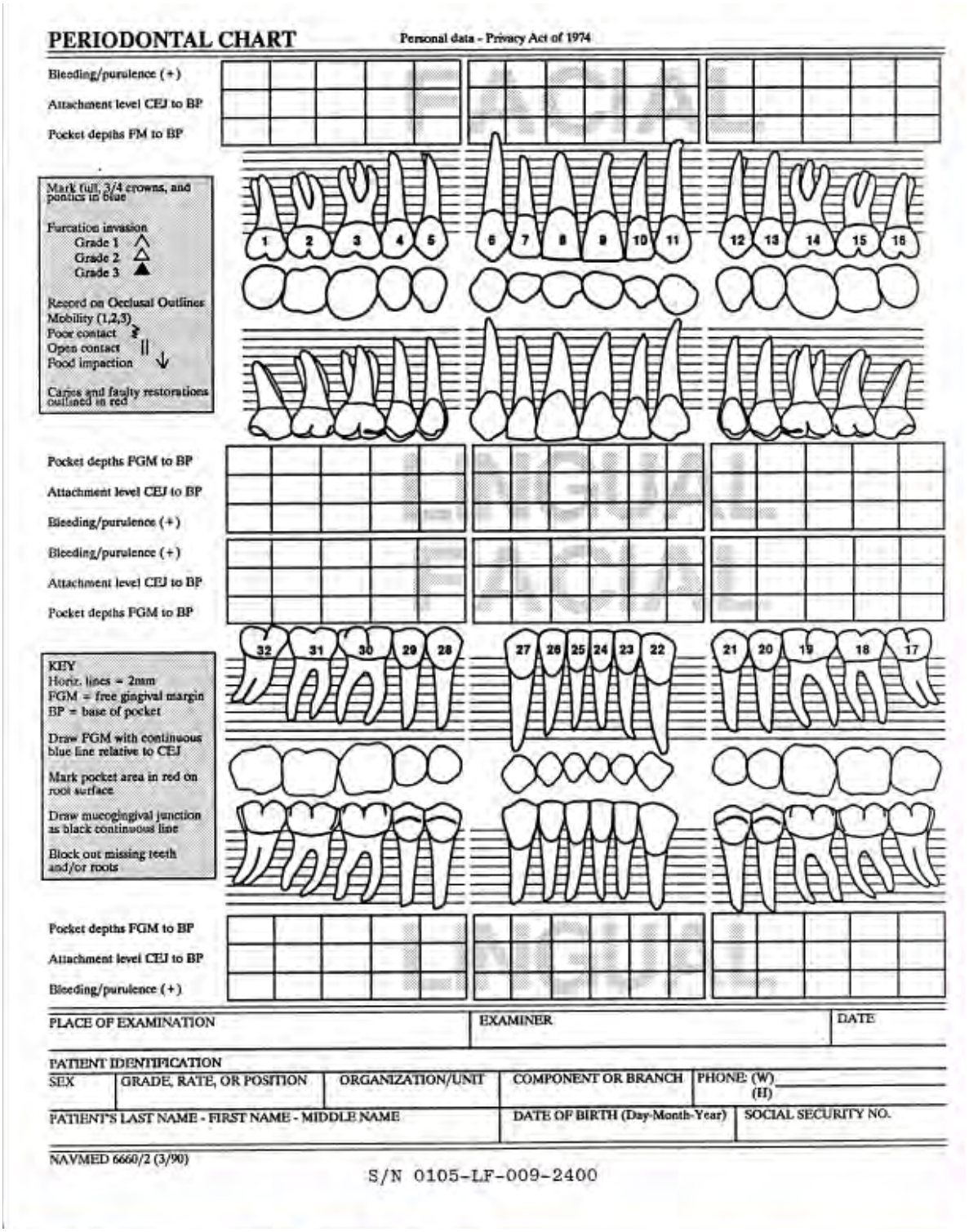
**Jennifer L. McGuire**

**LT, DC, USN**

**Primary Investigator**

**NPDS Periodontics Department**

**Appendix B2: Comprehensive Periodontal Charting Form**



PERIODONTAL CHART

Personal data - Privacy Act of 1974

Bleeding/purulence (+)
Attachment level CEJ to BP
Pocket depths FM to BP

Mark full, 3/4 crowns, and pontics in blue

Furcation invasion
Grade 1
Grade 2
Grade 3

Record on Occlusal Outlines
Mobility (1,2,3)
Poor contact
Open contact
Food impaction

Caries and faulty restorations outlined in red

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Pocket depths PGM to BP
Attachment level CEJ to BP
Bleeding/purulence (+)
Bleeding/purulence (+)
Attachment level CEJ to BP
Pocket depths PGM to BP

32 31 30 29 28 27 26 25 24 23 22 21 20 19 18 17

KEY
Horiz. lines = 2mm
FGM = free gingival margin
BP = base of pocket

Draw PGM with continuous blue line relative to CEJ

Mark pocket area in red on root surface

Draw mucogingival junction as black continuous line

Block out missing teeth and/or roots

Pocket depths FGM to BP
Attachment level CEJ to BP
Bleeding/purulence (+)

PLACE OF EXAMINATION | EXAMINER | DATE

PATIENT IDENTIFICATION

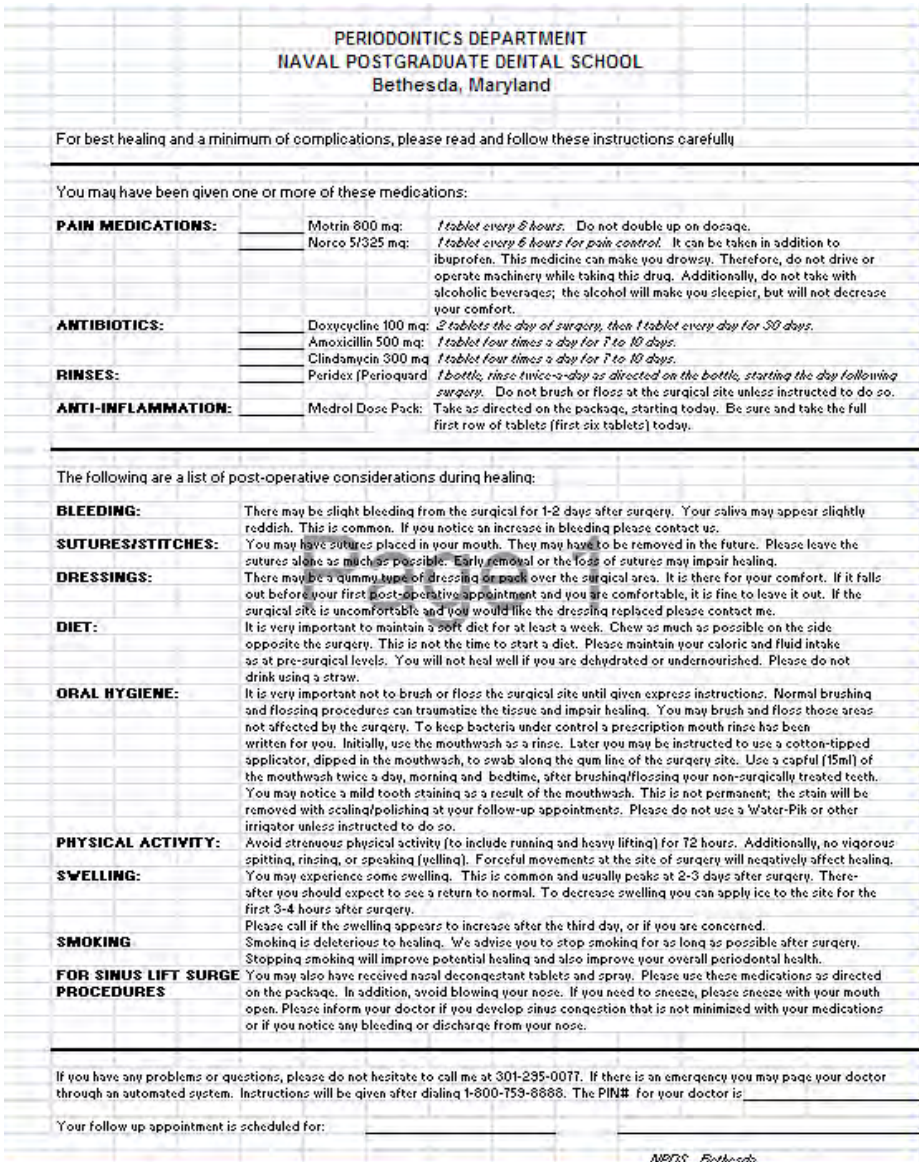
SEX | GRADE, RATE, OR POSITION | ORGANIZATION/UNIT | COMPONENT OR BRANCH | PHONE: (W) (H)

PATIENT'S LAST NAME - FIRST NAME - MIDDLE NAME | DATE OF BIRTH (Day-Month-Year) | SOCIAL SECURITY NO.

NAVMED 6660/2 (3/90)

S/N 0105-LF-009-2400

## APPENDIX B3: EXAMPLE OF NPDS PERIODONTICS DEPARTMENT POST-OPERATIVE INSTRUCTIONS



PERIODONTICS DEPARTMENT
NAVAL POSTGRADUATE DENTAL SCHOOL
Bethesda, Maryland

For best healing and a minimum of complications, please read and follow these instructions carefully

You may have been given one or more of these medications:

| | | | |
|---|---|---|---|
| **PAIN MEDICATIONS:** | _____ | Motrin 800 mg: | *1 tablet every 8 hours.* Do not double up on dosage. |
| | _____ | Norco 5/325 mg: | *1 tablet every 6 hours for pain control.* It can be taken in addition to ibuprofen. This medicine can make you drowsy. Therefore, do not drive or operate machinery while taking this drug. Additionally, do not take with alcoholic beverages; the alcohol will make you sleepier, but will not decrease your comfort. |
| **ANTIBIOTICS:** | _____ | Doxycycline 100 mg: | *2 tablets the day of surgery, then 1 tablet every day for 30 days.* |
| | _____ | Amoxicillin 500 mg: | *1 tablet four times a day for 7 to 10 days.* |
| | _____ | Clindamycin 300 mg | *1 tablet four times a day for 7 to 10 days.* |
| **RINSES:** | _____ | Peridex (Perioguard | *1 bottle, rinse twice-a-day as directed on the bottle, starting the day following surgery.* Do not brush or floss at the surgical site unless instructed to do so. |
| **ANTI-INFLAMMATION:** | _____ | Medrol Dose Pack: | Take as directed on the package, starting today. Be sure and take the full first row of tablets (first six tablets) today. |

The following are a list of post-operative considerations during healing:

| | |
|---|---|
| **BLEEDING:** | There may be slight bleeding from the surgical for 1-2 days after surgery. Your saliva may appear slightly reddish. This is common. If you notice an increase in bleeding please contact us. |
| **SUTURES/STITCHES:** | You may have sutures placed in your mouth. They may have to be removed in the future. Please leave the sutures alone as much as possible. Early removal or the loss of sutures may impair healing. |
| **DRESSINGS:** | There may be a gummy type of dressing or pack over the surgical area. It is there for your comfort. If it falls out before your first post-operative appointment and you are comfortable, it is fine to leave it out. If the surgical site is uncomfortable and you would like the dressing replaced please contact me. |
| **DIET:** | It is very important to maintain a soft diet for at least a week. Chew as much as possible on the side opposite the surgery. This is not the time to start a diet. Please maintain your caloric and fluid intake as at pre-surgical levels. You will not heal well if you are dehydrated or undernourished. Please do not drink using a straw. |
| **ORAL HYGIENE:** | It is very important not to brush or floss the surgical site until given express instructions. Normal brushing and flossing procedures can traumatize the tissue and impair healing. You may brush and floss those areas not affected by the surgery. To keep bacteria under control a prescription mouth rinse has been written for you. Initially, use the mouthwash as a rinse. Later you may be instructed to use a cotton-tipped applicator, dipped in the mouthwash, to swab along the gum line of the surgery site. Use a capful (15ml) of the mouthwash twice a day, morning and bedtime, after brushing/flossing your non-surgically treated teeth. You may notice a mild tooth staining as a result of the mouthwash. This is not permanent; the stain will be removed with scaling/polishing at your follow-up appointments. Please do not use a Water-Pik or other irrigator unless instructed to do so. |
| **PHYSICAL ACTIVITY:** | Avoid strenuous physical activity (to include running and heavy lifting) for 72 hours. Additionally, no vigorous spitting, rinsing, or speaking (yelling). Forceful movements at the site of surgery will negatively affect healing. |
| **SWELLING:** | You may experience some swelling. This is common and usually peaks at 2-3 days after surgery. Thereafter you should expect to see a return to normal. To decrease swelling you can apply ice to the site for the first 3-4 hours after surgery.<br>Please call if the swelling appears to increase after the third day, or if you are concerned. |
| **SMOKING** | Smoking is deleterious to healing. We advise you to stop smoking for as long as possible after surgery. Stopping smoking will improve potential healing and also improve your overall periodontal health. |
| **FOR SINUS LIFT SURGE PROCEDURES** | You may also have received nasal decongestant tablets and spray. Please use these medications as directed on the package. In addition, avoid blowing your nose. If you need to sneeze, please sneeze with your mouth open. Please inform your doctor if you develop sinus congestion that is not minimized with your medications or if you notice any bleeding or discharge from your nose. |

If you have any problems or questions, please do not hesitate to call me at 301-295-0077. If there is an emergency you may page your doctor through an automated system. Instructions will be given after dialing 1-800-759-8888. The PIN# for your doctor is _____

Your follow up appointment is scheduled for: _____ _____

NPDS Bethesda

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