LITERATURE REVIEW

PERIODONTAL ANATOMY

The tissues which surround the teeth, and provide the support necessary for normal function form the periodontium (Greek *peri-* "around"; *odont-*, "tooth"). The periodontium is comprised of the gingiva, periodontal ligament, alveolar bone, and cementum.

The gingiva is anatomically divided into the marginal (unattached), attached and interdental gingiva. The marginal gingiva forms the coronal border of the gingiva which surrounds the tooth, but is not adherent to it. The cemento-enamel junction (CEJ) is where the crown enamel and the root cementum meet. The Marginal gingiva in normal periodontal tissues extends approximately 2mm coronal tothe CEJ. Microscopically the gingiva is comprised of a central core of dense connective tissue and an outer surface of stratified squamous epithelium.

The space between the marginal gingiva and the external tooth surface is termed the gingival sulcus. The normal depth of the gingival sulcus, and corresponding width of the marginal gingival, is variable. In general, sulcular depths less than 2mm to 3mm in humans and animals are considered normal[1]. Ranges from 0.0mm to 6.0mm [2] have been reported.. The depth of a sulcus histologically is not necessarily the same as the depth which could be measured with a periodontal probe. The probing depth of a clinically normal human or canine gingival sulcus is 2 to 3 mm[2] [1].

Attached gingiva is bordered coronally by the apical extent of the unattached gingiva, which is, in turn, defined by the depth of the gingival sulcus. The apical extent of the attached

gingiva is the mucogingival junction on the facial aspect of the mandible and maxilla, and the lingual aspect of the mandibular attached gingiva. The palatal attached gingiva blends indistinctly with the similarly textured palatal mucosa.

Interdental gingiva occupies the interproximal space. It consists of a facial and a lingual papilla and the col. The col is a depression between the papillae which conforms to interproximal contact area[2]. It is sometimes absent when adjacent teeth are not in contact.

The epithelium of the attached gingiva and the outer surface of the marginal gingiva is a keratinized or parakeratinized stratified squamous epithelium. The sulcular epithelium, which extends from the coronal margin of the junctional epithelium to the coronal margin of the marginal gingiva, is a non-keratinized stratified squamous epithelium. This epithelium is thin and semipermeable, which may be important in the pathogenesis of periodontal disease as injurious bacterial products may pass into the gingiva and tissue fluids and humoral defense components may pass into the sulcus[3]. The junctional epithelium forms the collar of epithelial attachment around the tooth. It lines, and is bounded coronally by, the floor of the sulcus and is approximately 10 to 30 cells deep. It is widest coronally and tapers apically. It is normally attatched to both enamel and cementum at the CEJ, but may be located over enamel or cementum only, depending upon the stage of tooth development and the degree of gingival recession. The junctional epithelium has two distinct basal laminas. The *external basal lamina* is continuous with the basal lamina of the sulcular epithelium and attaches the junctional epithelium to the underlying connective tissue. The *internal basal lamina* attaches the junctional epithelium to the tooth surface. The apical aspect of the junctional epithelium is the germinative region. Cells migrate coronally and desquamate. The rate of desquamation of the junctional epithelium is greater than that of the sulcular or oral epithelium, which may be related to the maintenance of the junctional epithelium and the structure of the sulcus.

The junctional epithelium forms the attachment to the tooth surface, which keratinized cells cannot do. This may be important in repair to injured attachments. There are also fewer intercellular junctions in the junctional epithelium than oral or sulcular epithelium which may account for it's fragility and permeability to migrating cells and fluid[4].

Gingival connective tissue is termed the *lamina propria*, which consists of two layers. It is densely collagenous with few elastic fibers. The *papillary laye*r lies adjacent to the epithelium and consists of papillary projections which interdigitate with epithelial rete pegs. The *reticular layer* is contiguous with the periosteum of the alveolar bone. Gingival fibers are densely collagenous bundles of fibers with specific orientations and attachments. They are named according to their attachments or orientation. Their function is to provide for rigidity, structure and attachment of the gingiva[2].

The primary cellular element of the gingival connective tissue is the fibroblast. They provide for renewal and degradation of collagen and other constituents and are the primary regulators of gingival wound healing.

The periodontal ligament (PDL) surrounds the normal tooth root and forms the connective tissue attachment from the root to the alveolar bone proper. In addition to maintaining the tooth's attachment to the alveolar bone, and the structure of the gingiva in relation to the tooth, the PDL acts as a shock absorber and a means of transmitting occlusal forces to bone[2]. The cells of the PDL are active in ongoing remodeling of cementum and the PDL. They are active in the resorption and formation of collagen and cementum and the fibroblasts of the PDL may develop into cementoblasts and osteoblasts. Finally, the PDL

provides lymphatic drainage and blood vessels necessary for the nutrition of the cementum, bone , and gingiva[2].

The principal fibers of the PDL are densely collagenous and arranged in bundles, which insert into cementum and bone. Their terminal insertions are known as Sharpey's fibers. Electron microscopic evaluation has established a close association between these collagen fibers and fibroblasts. The principle fibers of the PDL are classified into five primary groups. These groups are, as for the gingival fibers, named for their location and orientation.

The *transseptal group* are interproximal fibers which insert into the cementum of adjacent teeth. They are a constant finding and will undergo reconstruction even when alveolar bone loss has occurred from periodontal disease[2].

The *alveolar crest group* insert into the cementum and alveolar crest apical to junctional epithelium. They function to retain the tooth in the socket by countering the coronal thrust of other ligaments[2].

The *horizontal group* fibers insert at right angles into the cementum and alveolar bone. Their function is as the alveolar crest group[2].

The *oblique group* is the largest of the groups. They run from the cementum in a coronal direction to insert on alveolar bone. They act to counter vertically oriented stresses[2].

The *apical group* fibers connect the bone at the fundus of the socket to the apical aspect of the root [2].

Cementum is the hard tissue which covers the tooth roots. It has a laminated arrangement and its' intercellular matrix is calcified. As cementum is formed, the fibers of the PDL are incorporated into it as Sharpey's fibers. There are two types of cementum: *cellular* and *acellular*. Cellular cementum is most abundant at the apex of the tooth. It is similar in structure to bone. Cementoblasts reside in lacunae and anastamose with one another through canaliculi. Unlike bone, cementum does not remodel. Its growth is by apposition and, as with bone and dentin, formation begins with an irregular meshwork of collagen fibers within a ground substance called *pre-cementum.* Acellular cementum forms a thin layer over the dentin surface of about 20-50 microns near the cervix to about 150 microns near the apex. Histologically this cementum appears clear. Its junction with the dentin is identified easily as the collagen fibers of the dentin are haphazardly arranged while those of the acellular cementum are regularly arranged and are roughly perpendicular to the cementum surface[5].

Cementum resorption is very common. It often does not involve the underlying dentin(70%) and may alternate with periods of regeneration[6]. There appears to be a predisposition to resorption at the cervical region which may be related to inadequate formation of pre-cementum[7]. Part of the process of continuous cementum deposition includes the formation of an uncalcified pre-cementum. This is thought to provide a barrier to apical migration of the junctional epithelium. The presence of epithelium in an area of resorption will preclude repair, therefore pathological periodontal pocket formation may be related to a defect in cementogenesis[8].

The alveolar process consists of the bone forming the alveoli. It may be anatomically divided into the *alveolar bone proper (cribriform plate*) and the *supporting alveolar bone*.

The components of the alveolar bone do not differ from bone elsewhere in the body. The alveolar bone proper consists of a thin layer of dense compact bone into which the Sharpeys fibers of the PDL insert deeply. Radiographically this bone appears as a thin radiopaque line surrounding the root called the lamina dura. The suppporting alveolar bone is comprised of the facial and lingual plates of the compact bone and cancellous trabeculae.

Alveolar bone is unique in its elevated rate of metabolism. Studies have shown that the metabolic rate exceeds that of diaphyseal bone[9]. It is in a constant state of flux with regard to height, contour, and density in response to forces exerted upon it. This high rate of metabolism may explain why alveolar bone is more severely affected by metabolic derangement's, such as renal osteodystrophy, and why minimal local factors may cause severe destructive changes in periodontal disease[5].

PERIODONTITIS - PATHOPHYSIOLOGY

Periodontitis refers to inflammation and destruction of the elements of the periodontium. Diseases of periodontal tissues are most commonly the result of an accumulation of plaque and calculus, and the proliferation of pathogenic organisms subgingivally within the sulcus. Occlusal trauma may also incite periodontal disease. Numerous systemic conditions also contribute to the development of periodontal disease. This discussion will be primarily focused on *chronic destructive periodontal disease* . This term is defined by periodontal disease caused by local factors, such as bacterial plaque accumulation, in otherwise healthy individuals [10]. Various classification schemes are present in the literature to describe this type of periodontitis, which essentially describe it's etiology and clinical morphology. General classifications include periodontitis caused by local plaque

accumulation, plaque accumulation and occlusal trauma, idiopathic juvenile forms, idiopathic adult periodontal atrophy, and periodontal atrophy from disuse.

Gingivitis is the inflammatory condition of the gingiva in which the junctional epithelium remains attached to the tooth root at its normal anatomical level. There are pathologic changes present, but no loss of periodontal attachment. Periodontitis occurs when pathologic changes progress to include the destruction of the periodontal ligament and migration of the junctional epithelium apical to the CEJ[11]. Periodontitis is always preceded by gingivitis, but gingivitis does not always progress to periodontitis.

Periodontal pocket formation occurs as a result of loss of periodontal attachment and pathologic deepening of the gingival sulcus[12]. True periodontal pockets are classified as suprabony (supracrestal) or infrabony pockets (intra-bony, subcrestal, intra-alveolar). In the former, the pocket floor lies coronal to the adjacent alveolar bone, while the floor of the latter lies apical to it. In the latter type the lateral pocket wall is bounded by the tooth surface and the alveolar bone. The pockets are lined by plaque covered cementum and enamel on one side, while the soft tissue walls and floor of the pocket are covered by a microulcerated layer of junctional epithelium, which is attatched to the root at the base of the pocket[13]. Increased sulcular depth may result from coronal displacement of the gingival margin due to enlargement of gingival tissue, apical migration of the junctional epithelium, or a combination of both. The process begins with inflammation of the connective tissues within the wall of the gingival sulcus[12]. As the normal sulcus progresses to a diseased periodontal pocket, the proportion of pathogenic microorganisms increases[14]. The microorganisms produce toxic products and cause inflammation, which results in tissue destruction and deepening of the sulcus[12]. With inflammation, the junctional epithelium lining the floor of the pocket is infiltrated with polymorphonuclear cells. When they

comprise greater than 60%, by volume, the integrity of the junctional epithelium is disrupted. Cellular enzymes degrade cellular junctions and the epithelium detatches from the tooth, causing further recession of the pocket[12]. Bony destruction is caused by microorganisms and their products, as well as the destructive effects of the immune products, such as prostaglandins and complement of the host, and substances from inflamed gingiva. Histologic evidence indicates that bone loss in chronic destructive periodontal disease occurs perivascularly. That is, the pattern of resorption roughly parallels the vascular tree of alveolar bone[13]. Osteoclastic resorption of alveolar bone results in the loss of attachment of the fibers of the (PDL).Chronic inflammation will result in alveolar bone loss at a rate of 0.2 to 0.3 mm per year[15]. Bone loss is due to active resorption by osteoclasts and mononuclear cells. It has been demonstrated that the degree of periodontal inflammation correlates positively with bone loss, and that the number of osteoclasts present in the alveolar bone crest is influenced by the proximity of the inflammatory infiltrate[16]. It is generally accepted that the increased number of osteoclasts and mononuclear cells is responsible for bone resorption. Increased vascularity accompanying inflammation may increase oxygen tension, which may enhance osteoclastic activity[17]. Plaque derived products are also thought to contribute to bone loss by direct and indirect means[18]. The exact mechenisms by which this occurs have not been defined, but possible pathways in which these products may act include: causing bone progenitor cells to differentiate into osteoclasts; stimulating the release of mediators from gingival cells which causes bone progenitor cells to differentiate into osteoclasts; direct destruction by noncellular mechanisms; stimulating the gingiva to secrete factors that destroy bone directly or which act as cofactors in bone destruction by other means[18].

Several mechanisms of collagen destruction have been described. It is believed that enzymes released from the inflammatory cells destroy interfibrillar crosslinkages within the

collagen fibers, which results in loss of collagen fiber stability. Other local conditions, such as increased temperature and acidity, may also play a role in fiber destruction[19]. Another proposed mechanism involves direct phagocytosis of intact collagen fibers. It has been shown in gingival tissue that the fibroblast is actively involved in collagen resorption[20]. Finally, it has also been proposed that the balance between normal collagen resorption and biosynthesis may be disrupted in the inflamed gingiva. Inflammatory cells may interfere with the fibroblasts ability to produce collagen[21]. These destructive changes are seen 0.5 to 1.0 mm apical to the apical extent of the pocket , while the structures further apical to this level remain unaffected[20].

GRADE III FURCATION DEFECTS

The majority of periodontal lesions demonstrate a favorable response to conventional periodontal therapy[22]. The degree to which periodontal destruction involves the furcation of multi-rooted teeth is commonly described by a grade assigned according to the classification system of Hamp *et al* .[23]:

Grade I - Horizontal penetration < 3mm..
Grade II - Horizontal penetration $\geq$ 3mm. , but not to the other side.
Grade III - Through and through horizontal penetration

Periodontal lesions extending into the furcation area of multi-rooted teeth, classified as Grade II or Grade III furcation defects, do not yield a consistent response to conventional periodontal therapy[23, 24]. In veterinary dentistry, Grade I and II furcation defects often go undiscovered on routine oral exams. If found they are often not treated due to expense or not being perceived as a clinical problem. It is often not until they have progressed to

Grade III defects that they are recognized as clinical problems and are, most commonly, treated by extraction. Grade III furcation defects present a common and challenging clinical problem in veterinary dentistry.

The application of techniques designed towards selective tissue regeneration has improved their prognosis, however Grade III defects remain the most challenging in human dental medicine[25, 26]. The size of the furcation defect has been shown to be directly related to the success of regenerative techniques. Lesions with furcation area entrances greater than 4.4 mm^2 showed incomplete regeneration, while areas less than 4.4 mm^2 frequently closed completely. A subsequent study in beagles demonstrated less successful regenerative therapy results when Grade III furcation defects were $\geq$ 5mm in height[27]. Treatment failures in this study were consistently associated with post-treatment recession of the gingival flaps. Other studies have suggested that defect size is of minor importance when compared to post treatment flap coverage of the defects[28, 29]. Subsequent studies have supported these findings[30].

GUIDED TISSUE REGENERATION (GTR)

Conventional periodontal therapy consists of scaling and root planing alone or in combination with periodontal surgery. Surgical procedures most often include gingivectomy, gingivoplasty, or some type of gingival flap procedure. The objectives of these procedures are enhanced tissue architecture and defect size reduction. When coupled with proper post-operative care this may delay or halt the progression of periodontal disease. These procedures, however, do not usually lead to the regeneration of periodontal support[31]. Periodontal regeneration is defined as restoration of the periodontal attachment apparatus, which includes periodontal ligament, cementum, and alveolar bone, and gingival

attachment. New attachment describes new cementum formation with inserting collagen fibers on a root previously denuded of it's periodontal ligament[32]. Periodontal regeneration is differentiated from new attachment in that it must include new bone formation. Ankylosis, or new attatchment without accompanying bone formation, does not represent true regeneration[33]. Many studies emphasize new attachment in their results[27,30,34]. While this type of healing is preferable to an epithelialized or ankylosed defect, it does not represent the normal anatomy and is less desirable than complete regeneration.

Studies have established that healing after conventional periodontal therapy includes the formation of a long junctional epithelial attachment[35], the extent of which has been shown to be largely determined in the first ten days of healing[36]. The re-establishment of the soft tissue-root interface depends upon the re-insertion of the regenerated principal fibers into the newly formed cementum[37]. Migration of the junctional epithelium into periodontal defects and the formation of sub-gingival plaque are thought to be the primary impediments to the re-establishment of normal connective tissue attachments[38]. It has been shown that granulation tissue from alveolar bone or gingival connective tissue is not capable of re-establishing normal periodontal attachments to the tooth root surface[39,40]. Previous studies have established that it is the cells from the periodontal ligament (PDL) that are responsible for the reestablishment of periodontal attachment[37,40-43]. Thus it appears that exclusion of the junctional epithelial cells from the treated root surface in the early stages of periodontal wound healing favors repopulation of the root surface by PDL cells, which favors the reestablishment of a normal connective tissue attachment[37,42,44]. The initial studies in humans and animals supported the concept that periodontal attachment could be predictably restored with GTR therapy[37,42,44]. The procedure involves placement of a barrier membrane which separates the exposed root surface and supporting alveolar bone

from the gingival tissue. The barriers prevent junctional epithelial cells and gingival connective tissue from colonizing the root surfaces, and provide space for selective repopulation of the root surface by the cells of the PDL. The first barriers used were made of expanded polytetrafluoroethylene (ePTFE)[1] or filter paper[2]. Subsequent studies have reported successful treatment of periodontal defects using ePTFE barriers in humans and dogs[30,45-47]. One disadvantage to the use of ePTFE and other non-resorbable barriers is the required re-entry procedure to remove them. For this reason bioresorbable barriers have been developed and studies have proven many of them to be as efficacious as ePTFE[22,30,34,48]. Resolut® bioresorbable barrier membrane is made of glycolide and lactide polymers. Membrane absorption is accomplished by hydrolysis and breakdown products are eliminated through the Krebs cycle as carbon dioxide and water. This process begins at 4-6 weeks and is completed by 8 months[49].

RADIOGRAPHY - CONVENTIONAL

In clinical patients, evaluation of the character and extent of regenerated tissue in the furcation has historically required a surgical re-entry procedure. Soft tissue probing measurements have been shown to be ineffective in quantifying healing of grade III furcation lesions[25]. Radiography serves as a non-invasive method in assessing osseous periodontal regeneration. However, there are a number of factors that limit the sensitivity of conventional radiography in assessing osseous changes in alveolar bone. Distortion of lesions due to positioning may exaggerate or understate their sizes. Also radiographs present a two-dimensional image of a three-dimensional structure. This allows overlying structures, such as cortical bone and tooth roots, to obscure defects. One study

[1] Gore-Tex Periodontal Material, W.L. Gore and Associates, Flagstaff, AZ.
[2] Millipore Corporation, Bedford, MA.

demonstrated that interproximal defects could not be identified on conventional radiographs if lingual and buccal cortical plates were intact[50]. The underestimation of infra-alveolar bone loss with conventional radiography has been attributed to it's inability to distinguish between the buccal and lingual alveolar crests[51]. In one report experimental interproximal lesions in dry skulls could not be documented on dental radiographs when the buccal and lingual cortical plates were intact[50]. For conventional radiography to accurately detect lesions in alveolar bone a change in bone mineral content of approximately 40% must occur[52,53]. Finally, radiographic technique and processing factors may influence the sensitivity of conventional radiographs.

RADIOGRAPHY - DIGITAL SUBTRACTION

Since the early 1980's subtraction radiography has been utilized as a means of assessing periodontal osseous changes[54]. Digital subtraction radiography involves a standardized radiographic image obtained prior to an anatomical change. This film is subtracted from a subsequent standardized radiograph as previously described[55,56]. Transformation matrix algorithms are applied which correct for geometric projection errors caused by changing the position of each film relative to the subject tissue. Image distortion caused by changes in the position of the x-ray source relative to the image plane cannot be corrected. Discrepancies in contrast and brightness can also be corrected. The final subtraction image derived is of the structure which has undergone change. The method of subtraction radiography in this study uses the subtraction image and the original film to perform volumetric quantification of defect size as previously described[57,58] and summarized below.

Prior to subtraction, serial sets of films are corrected for contrast and planar geometric discrepancies[58-60] using a program written in C language. Radiographs are digitized using a PC-Vision PLUS frame buffer (Imaging Technologies, Bedford, Massachusetts). Utilizing a morphologically aided technique[58,61] background noise is removed from the image and areas of bone gain or loss are isolated. The signed subtraction image is then converted to a binary image (black and white with no shades of gray) using an interactively controlled threshold. The threshold is adjusted until the area of bony change apears white. The binary image is then combined with the original subtraction image making the gray levels in the areas of change visible against the background. A statistical analysis of each feature determines the area of change. The aluminum reference wedge is used to determine the thickness of the wedge corresponding to the observed change in grey scale for the lesions. The mass of the lesion may then be calculated by multiplying the area X thickness X aluminum density X an aluminum to bone conversion factor. The lesion's volume is then calculated from the derived mass of the osseous change.

Numerous studies have demonstrated the superior sensitivity of subtraction radiography when compared to conventional transmission radiography in detecting small osseous defects[62]. It has been shown that while subtraction radiography could detect 0.5mm defects with nearly perfect accuracy, conventional radiographs did not achieve this level of sensitivity until the lesions were three times this size[63]. Subtraction radiography has demonstrated sensitivity sufficient to detect as little as 5% change in bone mineral content per unit volume[53,62]. Computerized digital subtraction radiographic techniques have demonstrated high sensitivity and specificity in assessing changes in bone height, bone density, and percentage of bone support around tooth roots[33,55,57]. One study

demonstrated specificity of 97.1% and sensitivity of 100% when hydroxyapatite chips, which were >12mg, were used to simulate lesions[64]. More recent studies have demonstrated the application of digital subtraction radiography as a tool for determining the size and mass of simulated periodontal lesions. When hydroxyapatite chips of known size were used to simulate osseous lesions, strong correlations (R^2=0.94) between the actual mass of the "lesion" and the mass calculated by digital subtraction radiography[55,65] were present. Further studies have been directed at validating techniques for the application of quantitative subtraction radiography for use in the clinical setting[53]. Increased sensitivity and the potential to provide quantitative data relative to osseous change makes digital subtraction radiography an important tool in the radiographic detection and assessment of periodontal lesions.

More recent studies have been directed at validating techniques for quantitative subtraction radiography to be used in clinical settings[53]. These studies have tested the ability of quantitative subtraction radiography to determine the size of hydroxyapatite chips of known density and weight. However, to the authors knowledge no studies have validated the capability of quantitative subtraction radiography to determine the volume of periodontal lesions and the volume of osseous regeneration subsequent to regenerative periodontal procedures.

FIBRIN GLUE - HISTORICAL BACKGROUND

One of the first reported uses of fibrin as a biomaterial was in 1909 when it was used as a degradable hemostatic agent[66]. Fibrinogen was first reported as an adhesive material in

1940, when it was used to anastamose experimentally severed sciatic nerves in rabbits[67]. The use of bovine thrombin in combination with fibrinogen was first reported in 1944[68]. The results of these early trials with fibrin glue were discouraging due to poor adhesive strength, possibly due to inefficient fibrinogen concentration techniques, and interest in fibrin glue waned for approximately 30 years[69,70].

The development of a more efficient method of fibrinogen concentration, cryoprecipitation, in 1972 yielded more promising results with fibrin adhesive systems[71] and initiated renewed interest in fibrin glue. Commercial tissue adhesive systems were developed as production methods were further refined. These commercial products are widely used currently, however, at present, they are only available in Japan and Europe[69].

Fibrinogen in commercial tissue adhesive systems is derived from pooled human plasma. Due to the potential risk of viral disease transmission, in particular hepatitis B and Human Immunodeficiency Virus, commercial tissue adhesive systems have been denied FDA approval in the United States. This has resulted in efforts to develop efficient and convenient methods of isolating concentrated fibrinogen/FXIII from single donors or autologous sources[72].

FIBRIN GLUE - PHYSIOLOGY OF HEMOSTASIS

A basic understanding of normal hemostatic mechanisms is necessary in order to understand the action of fibrin adhesives. For hemostasis to occur *in vivo* interactions between platelets, blood flow, coagulation factors, and the vasculature must occur [73].

Fibrin adhesives represent the end products of the common coagulation cascade, which explains their adhesive properties.

Fibrinogen is the primary protein precursor to the formation of blood clots. Thrombin, a serine protease, activates plasma fibrinogen to the fibrin monomer[69]. Fibrin monomers are arranged into progressively larger fibrils, and then fibers, in a three dimensional network. In the presence of calcium ions (Ca^{2+}), thrombin converts Factor XIII into its activated from (FXIIIa) by proteolytic cleavage. Factor XIIIa then converts the non-covalent bonds between fibrin monomers into covalent bonds by trans-amination, forming fibrin polymers[69]. Polymerization decreases the susceptibility of the clot to proteolytic digestion and increases its strength and stiffness. In the final phase the fibrin polymers are cross-linked into a dense mesh called the fibrin clot[69]. The covalent cross-linking of fibrin is also enhanced by the plasma proteins fibronectin and plasminogen, which also enhance adhesion of the clot to collagen substrates[69,74].

The fibrin clot is enzymatically degraded by plasmin[73]. The plasma protein plasminogen is enzymatically cleaved to form plasmin. Plasminogen may be activated by a number of endogenous enzymes such as urokinase, tissue plasminogen activator (tPA) and Factor XII, and exogenous enzymes such as streptokinase[69]. Plasmin itself can also act as an activator of plasminogen[69]. Antifibrinolytics block the conversion of plasminogen to plasmin or form complexes with the active site of plasmin to inhibit fibrinolysis. Endogenous antifibrinolytics include a_2-macroglobulin, a_2-antiplasmin, and antithrombin III. Exogenous antifibrinolytics include aprotinin and *e* -aminocaproic acid[69].

Based upon the normal physiologic coagulation cascade reactions, fibrin adhesive systems are classically comprised of two components. The primary ingredient in the first

component is concentrated fibrinogen along with FXIII, fibronectin, and other plasma proteins. The second component is thrombin. Various exogenous antifibrinolytic agents, such as aprotinin or *e* -aminocaproic acid, and ionized calcium (usually $CaCL_2$) have been added to thrombin to impede clot dissolution and enhance polymerization respectively[69 74]. When these two components are mixed a stable fibrin clot should form. It has been demonstrated that the application of autologous fibrinogen alone may be sufficient and possibly superior when fibrin glue is used to enhance tissue adhesion. In fact, in one study superior shear bonding strength was achieved using fibrinogen activated by endogenous thrombin alone, when applied to skin flaps[75].

FIBRIN GLUE - PERIODONTAL APPLICATION

Initial wound stability has been shown to be important to the healing periodontal wound[76]. Procedures designed to prevent gingival flap recession either by improved anchoring techniques[29], coronal repositioning[28], or wound stabilizing implants[76] have also proven beneficial in prevention of apical migration of junctional epithelium and enhanced new attatchment formation. Further studies have established the importance of early clot adhesion[77] on periodontal wound healing. Clot adhesion to the root surface by a fibrin linkage in the early stages of periodontal wound healing may be of primary importance in successful regeneration[78]. This adhesion may serve as a barrier to the apical migration of junctional epithelium[77-79]. The extent of epithelial migration has been shown experimentally to be largely determined in the first ten days of healing[36]. Various agents have been employed in the biomodification of root surfaces to enhance clot adhesion, such as fibronectin[47,80,81], stannous fluoride[82], citric acid[47,83], tetracycline[80], and heparin[77,81]. These agents have yielded varying degrees of success.

Commercially available tissue adhesives containing concentrated fibrinogen, fibronectin, and factor XIII[80,84-86,79] have been used as a means of promoting an early and stable bond between the gingival flap and the exposed root surface and for benefits provided to wound healing. Fibrin and factor XIII are known to promote fibroblast adhesion and multiplication[87]. The adhesive strength of fibrin glue has been shown to be proportional to its fibrin concentration[69]. Increased fibroblast growth and collagen production has been demonstrated with tissue adhesives providing enhanced early wound strength[88]. Also, by an as yet undefined mechanism, fibrin glues may have antibacterial properties as evidenced by studies on skin grafts in infected sites[89].

SUMMARY

Knowledge of the pathophysiology of periodontal disease provides the basis for therapeutic intervention aimed at arresting and reversing the resultant loss of periodontal attatchment. Procedures (GTR) designed to selectively guide the tissue elements involved in healing have been variously successful, as have attempts at biomodification of the root surface in concert with GTR procedures. Radiographic methods of evaluating periodontal defects represent a non-invasive means of assessing periodontal defects for treatment planning and monitoring of responses to periodontal therapy. Further validation of GTR techniques, and periodontal radiographic techniques, is required to determine the appropriate application of these modalities in clinical veterinary dentistry.

Accurate assessment of periodontal lesions, such as periodontal pockets and areas of alveolar bone loss, is important for diagnosis and treatment planning as well as monitoring

response to therapy. When within-patient comparisons are being made longitudinally, evaluating the healing or enlargement of a lesion, a relative measure may be sufficient. However, when data is compared between subjects or treatment modalities more exact quantification of the volume is necessary. Validation of the accuracy of radiographic techniques for the assessment of osseous regeneration would serve to eliminate the need for invasive, direct evaluation of osseous periodontal changes.

One purpose of the study reported here was to investigate the effects of autologous fibrinogen (fibrin glue) used alone or in combination with Resolut® barrier membrane on the periodontal healing of Class III furcation defects. A second objective of this study was to evaluate quantitative subtraction radiography as a non-invasive means of assessing periodontal regeneration.

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Periodontal healing of canine experimental grade III furcation defects treated with autologous fibrinogen and Resolut® barrier membrane.

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Abstract

Objective- To determine the effects of autologous fibrinogen and Resolut® barrier membrane on periodontal healing of canine experimental grade III furcation defects.

Animals- 18 conditioned, laboratory-source, adult Beagles.

Procedure- Defects were developed bilaterally at the second and fourth premolars and maintained for 12 weeks. Defects were treated with autologous fibrinogen, Resolut® barrier membrane, autologous fibrinogen and Resolut®, or debridement. Dogs received digital subtraction radiography, histopathologic, and histomorphometric analysis of defect healing at 1, 3, and 6 months post-treatment to determine: percent increase in defect bone volume, height, area, and length of periodontal regeneration along the perimeter of the defect.

Results- Comparisons at post-treatment intervals indicated significantly ($P < 0.05$) greater healing of debridement and autologous fibrinogen treated defects at 3 months, however by 6 months there were no significant differences in defect healing for all histomorphometric parameters. Defects receiving Resolut® were associated with significantly less root ankylosis. Defects receiving debridement had significantly greater increases in bone volume at 6 months post-treatment compared with groups receiving Resolut®. There was a significant correlation between regenerated bone area, bone volume, and periodontal regeneration for all treatments at 3 and 6 months post-treatment.

Conclusion- Autologous fibrinogen and Resolut® barrier membrane did not enhance the amount of periodontal healing compared with debridement only. However, Resolut® treated defects were essentially absent of root ankylosis.

Clinical Relevance- Canine periodontitis causing grade III furcation involvement may respond equally well to conservative periodontal surgery compared with guided tissue regenerative techniques. However, the prevention of root ankylosis is a substantial benefit favoring this latter methodology.

Periodontal tissues such as the gingiva, periodontal ligament (PDL), and alveolar bone provide tooth support and an anatomic defense mechanism against bacterial entrance to the furcation. Classification of furcation involvement indicates severity of periodontal attachment loss and provides guidelines for appropriate management and prognosis. Attachment loss at the furcation entrance indicates grade I classification. Grade II involvement extends under the roof of the furcation, but is not a through-and-through defect. A complete, through-and-through defect under the roof of the furcation is grade III involvement[1]. This latter grade is associated with a poor prognosis for long-term tooth maintenance based on horizontal bone loss through the entire furcation and vertical bone loss apically along tooth roots, which is indicative of destructive periodontitis[1].

One of the purposes of periodontal treatment is the regeneration of bone and ligamentous attachments that have been destroyed by disease. Conventional periodontal therapy consists of scaling and root planing alone or in combination with periodontal surgery. Surgical procedures most often include gingivectomy, gingivoplasty, or some type of gingival flap procedure. These procedures may delay or halt the progression of periodontal disease when combined with appropriate post-treatment oral hygiene. These procedures, however, do not usually lead to complete regeneration of periodontal supporting tissues[2].

Failure of periodontal treatment is often related to apical migration of junctional epithelium[3]. The presence of junctional epithelium in periodontal defects and the formation of sub-gingival plaque are thought to be the primary impediments to the re-establishment of normal periodontal connective tissue attatchment[4]. Previous studies have established that cells from the PDL are responsible for the re-establishment of periodontal attatchment[5-9]. Thus it appears that exclusion of the junctional epithelial cells from the treated root surface

in the early stages of periodontal wound healing favors repopulation of the root surface by PDL cells, promoting periodontal regeneration[6, 8, 10].

Studies in humans and animals support the concept that periodontal attatchment can be predictably restored with guided tissue regeneration (GTR) therapy[6,8,10]. This procedure involves placement of a barrier membrane which separates the exposed root surface and supporting alveolar bone from the gingival tissue. GTR prevents junctional epithelial cells and gingival connective tissue from colonizing root surfaces, allowing space for selective repopulation of the root surface by the cells of the PDL.

The first barriers used were made of expanded polytetrafluoroethylene (ePTFE) or filter paper. Subsequent studies have reported successful treatment of periodontal defects using ePTFE barriers in humans and dogs[11-14]. One disadvantage to the use of ePTFE and other non-resorbable barriers is the re-entry procedure required for removal. For this reason, bioresorbable barriers have been developed and studies have proven many of them to be as efficacious as ePTFE[14-17].

Intrinsic wound healing characteristics also influence periodontal regeneration. Clot adhesion to the root surface by a fibrin linkage in the early stages of periodontal wound healing may be of primary importance in successful regeneration[18,19]. This adhesion may serve as a barrier to the apical migration of junctional epithelium[18-20].

The purpose of this study was to determine the effects of absorbable Resolut® barrier membrane (RES) and autologous fibrinogen (AF) on healing of severe, experimental grade III furcation defects in dogs.

Materials and Methods

Experimental defect preparation- Eighteen mature Beagles, weighing 10.0 ± 1.9 kg received bilateral mandibular premolar furcation defects. Prior to defect creation the subject teeth were evaluated for the presence of plaque, calculus, or gingivitis. Teeth were examined for the presence of periodontal pockets using a periodontal probe. Dogs were approximately 2-years-old and had been used previously in a vaccination efficacy research study. Under general anesthesia full-thickness mucogingival flaps were elevated on the lingual and buccal aspect of the mandibles with vertical relief incisions at the distal line angle of the first premolar and the mesial line angle of first molar. Grade III furcation defects were surgically created bilaterally at the P2 and P4 using a combination of instruments including a diamond bur on a slow speed handpiece, bone chisels, and periodontal curettes. The alveolar bone, periodontal ligament (PDL), and cementum were removed from the furcation areas similar to other experimental protocols[14,21,22]. The height of the resulting defects was 5 mm as measured from their apical base to the furcation fornix. Furcation defects were maintained by placement of dental impression material[i] within the defect[14,21]. The flaps were replaced to their original position and sutured with 4-0 polyglactin 910[ii] using simple interrupted and interdental patterns. Analgesia was provided using butorphanol tartrate[iii] (0.4 mg/kg SQ QID) for the first week following defect preparation. The impression material was removed 6 weeks following implantation using ketamine[iv] (5mg/kg IV) and acepromazine[v] (0.05mg/kg IV) for sedation.

Furcation defect treatments- Two-weeks prior to furcation defect treatment, a complete dental scaling and polishing was performed in each dog, followed by daily irrigation of the mandibular arcades with 0.12% chlorhexidine gluconate[vi]. Grade III furcation defects were present in all cases at the time of repair. All juxtaposed gingiva had mild to moderate inflammation with abnormal apical migration of sulcar epithelium. Full-thickness

mucogingival flaps were elevated as described previously 12-weeks following furcation defect preparation. All defects were thoroughly debrided using an ultrasonic scaler and periodontal curettes until all granulation tissue was removed. Periodontal pocket epithelial debridement was performed using periodontal curettes and a #15 scalpel blade. Reference notches, for subsequent histomorphometric analysis, were made on the interradicular surface of each root at the level of the base of the defect. A split mouth experimental design using the mandibular second [P2] (n=35) and fourth [P4] (n=35) premolars was used[23,24]. The third premolar was designated as a P2 in 2 dogs having congenital absence of one of the experimental teeth. Periodontal treatments were randomly assigned in each dog as follows: (A) Autologous fibrinogen [AF]; (B) AF and Resolut®[vii] [AF/RES]; (C) Control (debridement only)**;** and, (R) RES only.

Concentrated autologous fibrinogen was obtained using the ethanol precipitation technique[25,26]. Ethanol precipitated autologous fibrinogen (0.1ml) was used in groups A and B just prior to suturing the mucogingival flaps, while the lingual flap was digitally maintained in apposition to the tooth to prevent fibrinogen leakage (Fig.1). The fibrinogen was placed within the furcation either alone or between RES. No external source of thrombin was utilized. Activation of fibrinogen to fibrin was dependent upon endogenous thrombin from hemorrhage at the treatment site. A minimum of one ml of concentrated AF was saved for individual fibrinogen level quantitation, which was performed on each sample within 8 hours of preparation.

RES was contoured for each tooth, based on manufacturers recommendations, and applied bilaterally. RES was cut to overlap the cemento-enamel junction by 1mm, the mesial and distal defect borders by 1-2 mm, and the apical border by 3mm. RES was secured with RES absorbable sutures placed in a sling pattern (Fig.1). The buccal and lingual

mucogingival flaps in all groups were repositioned 3-4 mm coronal to the cementoenamel junction using preplaced ePTFE[viii] sutures in an interdental mattress pattern (Fig.1). Vertical relief incisions were apposed using ePTFE in a simple interrupted pattern. ePTFE sutures were removed 7 - 10 days post-treatment. Doxycycline[ix] (2.0 mg/kg PO BID) was administered for the first three post-treatment weeks. Analgesia was provided using butorphanol tartrate (0.4 mg/kg SQ QID) for the first post-treatment week. A 0.12% solution of chlorhexidine was applied as an oral lavage for four weeks following treatment in attempt to decrease plaque accumulation on treated teeth. Soft food[x] was fed throughout the study and water was provided ad libitum. Six dogs each were euthanatized by barbiturate[xi] overdose at 1, 3, and 6 months following furcation defect treatment. The study design was approved by the Institutional Animal Care and Use Committee (IACUC).

Imaging procedures- Dental radiographs of the mandibular premolars in each dog were made prior to furcation defect formation, immediately prior to defect treatment, and at euthanasia. Polymethylmethacrylate[xii] was used to form an impression of the maxillary premolars and canines, and the mandibular canines and molars, within which an intraoral radiographic film mount was imbedded. The intraoral mounts were then attatched to the ring bracket and cone of a dental radiograph unit (Fig.2). These customized mounts were used for all subsequent radiographs to maintain uniformity of magnification and angulation between dental radiographs in each dog. An aluminum wedge of known dimensions was placed on the film mount to be superimposed on the radiograph and serve as a density reference for subtraction radiography. Measurements were used to calculate % changes in furcation defect bone volume(BV). Subtraction radiography was performed as previously described[27-30].

Histopathologic & histomorphometric analysis- Under general anesthesia, 10 minutes prior to euthanasia, dogs were heparinized with 5cc of 1:1000 sodium heparin administered intravenously. Immediately following euthanasia the heads were removed and perfused with approximately 300-500cc 10% neutral buffered formalin (NBF) via carotid infusion**.** The treated premolars were then removed en bloc from the mandibles, fixed in 10% NBF, and demineralized. Complete demineralization was confirmed radiographically. The specimen blocks were washed, dehydrated in graded alcohol solutions, cleared with xylene, and infiltrated/embedded in parafin. Seven μM thick serial sections were made in a mesio-distal plane, parallel to the long axis of the root. The section exhibiting the widest area of pulp cavity was considered representative of the mid-furcation. The sections were processed routinely and stained with hematoxylin, phloxine, and eosin.

Mid-furcation sections were then digitized and stored on a computer. Initial defect and regenerated tissue dimensions were determined using measurement software[xiii]. For measurement purposes, the defect base was delineated by the apical portion of the interradicular notches, and the coronal extent by the furcation fornix. Where notches were not visible the defect base was easily identified by the disruption of normal cementum and dentin. The mean value of 3 separate measurements for each defect parameter measured was used for all histomorphometric calculations**.** Measurements (mm or mm^2) of defect parameters enabled calculations including: % maximum bone height (BH) gain; % defect bone area (BA) gain; % gain in length of periodontal regeneration (PR) which included PDL-like attachment, cementum, and bone along the perimeter of the defect.

Statistical analysis- Mean defect area measurements of the 2nd and 4th premolars were compared using a Student's *t* -test for paired data. The concentration of fibrinogen was determined for each animal. Differences between these concentrations for each group were

compared using one-way analysis of variance. Comparisons of percentage gains between groups for BH, BA, and PR were analyzed with analysis of variance for a randomized block design. The relationship between percent BA gain and percent BV gain, and percent gain in PR, were assessed using Pearson's correlation coefficient and regression analysis. A Chi-squared test for independence was applied to evaluate the relationship between membrane use and the development of root ankylosis. Statistical analysis was performed using a commercially available statistical software program[xiv]. Statistical probability of $P < 0.05$ was considered significant.

Results

Dogs had no clinical or radiographic signs of dental disease of the mandibular premolars prior to furcation defect preparation. Concentrated fibrinogen values (mg/dL) for 1 (7.6^3 +/- 2.5^3), 3 (5.8^3 +/- 3.7^3), and 6 (7.3^3 +/- 2.5^3) month groups were not significantly different. Mid-furcation defect surface areas (mm^2) of P2 (8.2 +/- 1.6) compared with P4 (8.9 +/- 1.5) were not significantly different. The mean defect height for all teeth at the time of treatment was 3.9 $\pm$ 0.4 mm. Mucogingival flaps healed with minimal associated inflammation and exhibited varying degrees of post-treatment flap recession (Fig. 3).

1-month observations- Healing furcation defect lesions were characterized by exuberant, immature connective tissue proliferation (Fig. 4). Three of 24 teeth had substantial bony healing within the defect and each exhibited ankylosis at the fornix of the furcation. One of the three teeth exhibiting ankylosis received RES. Superficial root resorption was not present in any specimens in association with ankylosis, but was present in one tooth adjacent to fibrous connective tissue. When present, new PDL-like tissue was wider, less organized, and oriented parallel to the root surface compared with uninterrupted PDL (Fig. 4). RES fibers were observed within the healing defects of teeth receiving treatments R and B (Fig. 4).

The parameter of % increase in BH for treatments A (25.2 $\pm$ 37.5), B (21.5 $\pm$ 38.6), C (40.9 $\pm$ 38.8), and R (21.1 $\pm$ 10.7) were not significantly different. The parameter of % increase in BA for treatments A (14.8 $\pm$ 30.9), B (15.5 $\pm$ 32.7), C (28.3 $\pm$ 35.6), and R (11.4 $\pm$ 9.2) were not significantly different. The parameter of % increase in PR for treatments A (14.5 $\pm$ 22.1), B (12.9 $\pm$ 28.4), C (21.0 $\pm$ 18.8), and R (11.2 $\pm$ 11.3) were not significantly different. The % change in furcation defect BV for treatments A (25.9 $\pm$

29.1), B (15.4 ± 26.6), C (26.1 ± 55.1), and R (7.9 ± 14.2) were not significantly different. There was no correlation between % change in furcation defect BV and BA.

3-month observations- Furcation defect bony healing had increased and matured resembling lamellar cancellous bone. Areas of ankylosis were noted, primarily towards the fornix of the furcations, in 8 of 24 teeth (Fig. 5). Only 1 of these 8 teeth received RES. Junctional epithelium was present at the coronal aspect of the furcation in 14 of 16 furcation defects showing partial bony healing. One of 2 teeth with moderate superficial root resorption had associated ankylosis. New PDL-like tissue also appeared more mature and organized. Differences compared with normal PDL included decreased fibrocyte organization with oblique orientation, and thin filamentous nuclei.

The parameter of % increase in BH for treatments A (80.1 ± 24.1) and C (72.1 ± 38.4), was significantly different compared with treatments B (43.0 ± 36.9) and R (43.7 ± 20.3). The parameter of % increase in BA for treatment A (76.8 ± 28.7) was significantly different compared with treatments B (28.1 ± 27.2), C (50.3 ± 32.8), and R (28.4 ± 15.6). The parameter of % increase in PR for treatment A (60.7 ± 26.6) was significantly different compared with treatments B (35.7 ± 30.0) and R (34.6 ± 17.2), however there was no significant difference between treatment C (48.6 ± 31.4) and treatments A, B, or R. The % change in furcation defect BV for treatments A (34.3 ± 29.7), B (9.9 ± 11.2), C (31.3 ± 19.1), and R (30.8 ± 14.3) were not significantly different. The % change in furcation defect BV was positively correlated with BA.

6-month observations- The furcation defects again demonstrated histologic evidence of advanced healing marked by mature lamellar bone within the defect and fibrous connective tissue resembling normal PDL (Fig. 6). Ankylosis was noted in 10 of 24 teeth and also tended to occur towards the fornix of the furcations. Ankylosis involved ≥ 30 % of the

mid-furcation root surface length in 5 of these teeth. Root resorption was juxtaposed to areas of ankylosis in 9 of 10 teeth and 4 of these exhibited extensive replacement resorption (Fig. 7). The remaining tooth received RES and exhibited mild ankylosis and no associated root resorption. Two furcation defects had healed with fibrous connective tissue to the level of the furcation fornix with no evidence of junctional epithelium or root resorption. The remaining 12 furcation defects showed incomplete bony healing and the coronal aspect of the furcations had inflamed junctional epithelium.

The parameter of % increase in BH for treatments A (70.4 $\pm$ 39.0), B (55.7 $\pm$ 25.9), C (64.4 $\pm$ 47.9), and R (50.9 $\pm$ 28.9) were not significantly different. The parameter of % increase in BA for treatments A (53.8 $\pm$ 39.6), B (34.2 $\pm$ 18.5), C (56.3 $\pm$ 46.6), and R (28.3 $\pm$ 19.2) were not significantly different. The parameter of % increase in PR for treatments A (49.4 $\pm$ 27.5), B (47.2 $\pm$ 27.0), C (29.3 $\pm$ 28.8), and R (33.3 $\pm$ 22.4) were not significantly different. The % change in furcation defect BV for treatment C (76.6 $\pm$ 36.3) was significantly different compared with treatments B (28.0 $\pm$ 18.3) and R (15.6 $\pm$ 9.7), however there was no significant difference between treatment A (55.0 $\pm$ 33.3) and treatments B, C, and R. The % change in furcation defect BV was positively correlated with BA.

Overall, teeth receiving RES (n=36) had significantly less histopathologic evidence of root ankylosis (8.3 %) compared with non-RES (n=36) treatments (50%) which were evenly distributed in groups A (n=10) and C (n=8).

Discussion

Our study was designed to address the aforementioned problems associated with periodontal wound healing by evaluating the individual and combined effects of a root surface biomodifier (concentrated AF) and a resorbable barrier membrane (RES). A split-mouth design was used to allow development and treatment of symmetric lesions of sufficient number to assess all treatments in each dog[23]. AF and RES treatments were localized to either a P2 or P4, with the third premolar acting as a non-treated intermediate in all but 2 dogs. This makes spill-over effects unlikely and further supports this design[24].

Commercially available tissue adhesives containing concentrated fibrinogen, fibronectin, and factor XIII have been used to generally benefit wound healing and to promote an early and stable bond between the mucogingival flap and the exposed root surface[20,31-34]. Fibrin and factor XIII are known to promote fibroblast adhesion and multiplication[35]. Tissue adhesives have been shown to provide early wound strength due to increased fibroblast growth and enhanced collagen production[36]. The adhesive strength of fibrin has been shown to be proportional to its concentration and not necessarily related to the addition of exogenous thrombin[37]. In fact, superior shear bonding strength has been achieved using fibrinogen activated by endogenous thrombin[xv] . In this study, conversion of AF to fibrin was dependent upon endogenous thrombin.

RES is made of glycolide and lactide polymers. Membrane absorption is by hydrolysis and breakdowm products are eliminated through the tricarboxylic acid cycle as carbon dioxide and water. This process begins at 4-6 weeks and is completed by 8 months[38]. In this study RES fibers were seen only in the 1 month specimens. Resorption was essentially

complete by 3 months. Several authors have stated the importance of the timing of membrane dissolution to correspond with selected cell repopulation[39,40]. It has been shown that apical migration of epithelial cells is primarily within the first ten days following treatment[41], and that mitotic activity of PDL cells decreases 3 weeks post-treatment with coronal migration peaking at 1 to 2 weeks. In light of the sequence of early healing events it appears that 3-4 weeks is the most reasonable time to maintain the membrane structure[42]. If RES remains beyond these time frames, it could potentially exert a negative effect on early bone and cementum regeneration[43].

The size and chronicity of the grade III furcation defects developed and evaluated in this study reflect a common and challenging clinical problem in veterinary dentistry. The size of the furcation defect has been shown to be directly related to the success of GTR therapy. In one study defects less than 2mm in apico-coronal height consistently regenerated and healed, while defects with heights greater than 3mm failed to exhibit complete healing[22]. Another study in beagles demonstrated less successful GTR results when grade III furcation defects were $\geq$ 5mm in height[22]. In our study, defects had a mean area of 8.7 $\pm$ 1.8mm^2 and mean heights of 3.9 $\pm$ 0.4mm at the time of treatment. Chronic periodontal defects similar in size and duration to those described here have been associated with a limited capacity for spontaneous healing[21]. Treated acute furcation defects may be associated with more consistent and complete connective tissue attachment. However, an experimental model evaluating chronic furcation defects more accurately reflects clinical conditions present in naturally occuring periodontal disease in dogs and humans[21]. Chronic defects generally have incomplete healing characterized by long junctional epithelium, gingival recession, and less connective tissue repair[44].

Contraction and recession of the mucogingival flap has been associated with treatment failure[14,44-46]. Mucogingival flap management in this study was designed to avoid the

complication of gingival recession and premature exposure of the treated furcation defect. Flaps were coronally repositioned in all treatment groups to provide soft tissue coverage of the defect during the early phases of wound healing. Mucogingival flap healing in this study was uncomplicated and likely did not influence our results.

Periodontal regeneration is defined as restoration of the periodontal attatchment apparatus, which includes periodontal ligament, cementum, alveolar bone, and gingiva. New attatchment describes new cementum formation with inserting collagen fibers on a root previously denuded of it's periodontal ligament[47]. Periodontal regeneration is differentiated from new attatchment in that it must include new bone formation. Ankylosis or new attatchment in the absence of bone formation does not represent true regeneration[48]. Ankylosis or replacement resorption in experimental models may occur following the creation of bone defects secondary to increased granulation tissue production. However, this condition rarely occurs spontaneously[42,49]. Many studies have emphasized new attatchment in their results[14,15,22]. While this type of healing is preferable to an epithelialized or ankylosed defect, it does not represent normal anatomy and is less desirable than complete regeneration. In this study, new attachment only was not considered a positive result.

Evaluation methods including histopathologic and histomorphometric analysis, and digital subtraction radiography were used to evaluate periodontal regeneration in this study. The mid-furcation region was considered the most representative area to assess periodontal regeneration. Histopathologic and histomorphometric analysis provided evidence of specific cellular activity and quantitation of periodontal regeneration in this limited, one-dimensional area. The results in the 6-month group likely best represented the long-term results of the treatments used in this study. Studies have shown that cementogenesis

peaks at approximately 3 months[50]. It has been recommended to perform histopathologic evaluation of regenerated cementum after a minimum of 6 months post-treatment to allow for variability in individual healing responses[48]. New bone and periodontal ligament formation are thought to be independent events[42]. In this study, areas of new cementum formation were seen in the absence of new alveolar bone, which is consistent with previous observations[8,51]. However, in general, areas of bone adjacent to the root surfaces, in the absence of ankylosis, demonstrated new cementum formation and a positive correlation was shown between new BA and PR.

Small amounts of ankylosis may occur after damage to the periodontal ligament, however it may be reversible if less than 20% of the root surface is affected[52]. Larger amounts of ankylosis are an undesirable result with periodontal treatment and may lead to replacement of dentin by bone, pulp death, and, eventually tooth loss[52]. It is generally accepted that if migrating bone cells contact a curetted root surface ankylosis and root resorption will result[53,54]. Although these complications rarely occur spontaneously in the clinical setting, they do occur commonly in the presence of active granulation tissue from bone as occurs in the creation of artificial defects[42]. A commonly utilized method of defect creation was used in this study[14,21,22] which includes aggressive root planing in which the cementum is removed. Although our methodology may have contributed to ankylosis formation[52], it is imperative to debride the cementum, which is laden with bacteria and endotoxin, when treating furcal defects with root exposure[55]. Another factor which may influence the incidence of root ankylosis is the observation that the rate of alveolar bone healing may exceed that of the PDL[54]. Ankylosis developed in 13 of 14 experimental furcation defects $\geq$ 4.3mm in vertical depth and bone formed >5mm from the defect base demonstrated a 90% frequency of ankylosis. Ankylosis was attributed to bone proliferation

in advance of the coronal migration of cells from the PDL[45]. These findings are consistent with the pattern of ankylosis formation observed in this study. We observed ankylosis in all treatment groups, especially at 3 and 6 months post-treatment. Ankylosis associated replacement resorption was seen most frequently at 6 months. In teeth receiving RES, only three developed ankylosis, which was minimal (< 20%) in all cases and free of associated root resorption.

Application of AF as described in this study did not improve long-term periodontal defect healing. A significant increase in BA, BV, and PR noted at 3 months following AF treatment may have been secondary to enhanced wound stability or other wound healing properties of AF. However, since all other treatment groups had improved results by 6 months, it is likely that any early wound healing attributes related to AF were transient and failed to provide the long term benefits of selective cell exclusion**.** A previous report has indicated that bone and cementum regeneration was suppressed when comparing implanted sites to controls when using polylactic acid implants for wound stabilization[43]. This result may have been an effect of membrane associated inflammation and/or the slowly degrading membranes acted as physical barriers to cells from the PDL or alveolar bone[43]. These effects may explain the relative delay in osseous regeneration and attatchment gain in teeth receiving RES.

In clinical patients, evaluation of the character and extent of regenerated tissue in the furcation has historically required a surgical re-entry procedure. Soft tissue probing measurements have been shown to be ineffective in quantifying healing of grade III furcation lesions[56]. Radiography serves as a non-invasive method in assessing osseous periodontal regeneration. Validation of the accuracy of radiographic techniques for the assessment of osseous regeneration would serve to eliminate the need for invasive, direct evaluation of osseous periodontal changes. Computerized digital subtraction radiographic

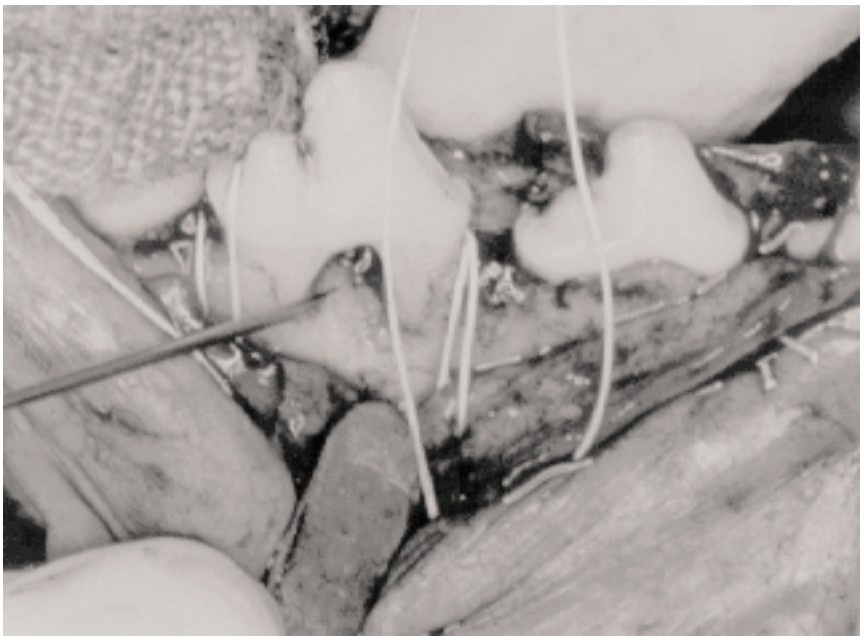
techniques have been proven effective in assessing changes in bone height, bone density, and percentage of bone support around tooth roots[48]. To our knowledge digital subtraction radiography has not been used to assess the volume of osseous regeneration following GTR procedures. The mid-furcation location for assessment is considered representative of healing activity within the periodontal defect, and therefore, it might be expected that there would be a positive correlation between BA and the BV as measured by subtraction radiography. Our results demonstrated a significant correlation between BA and BV as measured by subtraction radiography. This correlation was not established for the one month group, where osseous regeneration was minimal. Perhaps the volumetric assessment is a more representative measure of osseous regeneration when it is minimal because the entire defect is measured versus a one dimensional histologic section. Minimal regeneration may be accompanied by non-uniform coronal bone growth. As regeneration increases, bone distribution through the defect may become more uniform making for more consistent correlations between histologic sections and volumetric measures. Regenerated BV was superior in the control group 6 months post-treatment compared with the results of histomorphometric analysis. Volumetric analysis based on digital subtraction radiography may be expected to be superior to a one-dimensional, histologic, mid-furcation assessment with respect to osseous healing within the periodontal defect. However, further study is required to quantify and associate actual increases in defect BV with BV determined by digital subtraction radiography.

Historically, therapy for grade III furcation defects of the severity described in this study has warranted a guarded to poor prognosis for periodontal regeneration, regardless of treatment. Although treatments including RES essentially prevented ankylosis, A and C groups showed greater, albeit not significant, trends in periodontal regeneration based on histomorphometric and radiographic analysis. Greater periodontal healing may have been achieved with GTR if intensive post-treatment oral hygiene had been used. The post-

treatment oral hygiene protocol in other studies has ranged from antibiotics and soft food only to regular scaling, oral antibiotics, nonsteroidal anti-inflammatory drugs, and oral chlorhexidine rinses[15,57]. Postoperative wound care in this study was limited to chlorhexidine rinses and oral antibiotics. We consider this to represent a realistic level of post-treatment care that could be anticipated in veterinary dental practice. This observation is supported in a recent 6-month study in which a dedicated population of pet owners had surprisingly low (53 %) post-treatment compliance with oral hygiene recommendations, defined as brushing their pet's teeth several times weekly[58]. Although the benefits of regular scaling, polishing, and toothbrushing in managing periodontitis have been clearly demonstrated[59], difficulty and expense are factors that limit their application in veterinary dental practice.

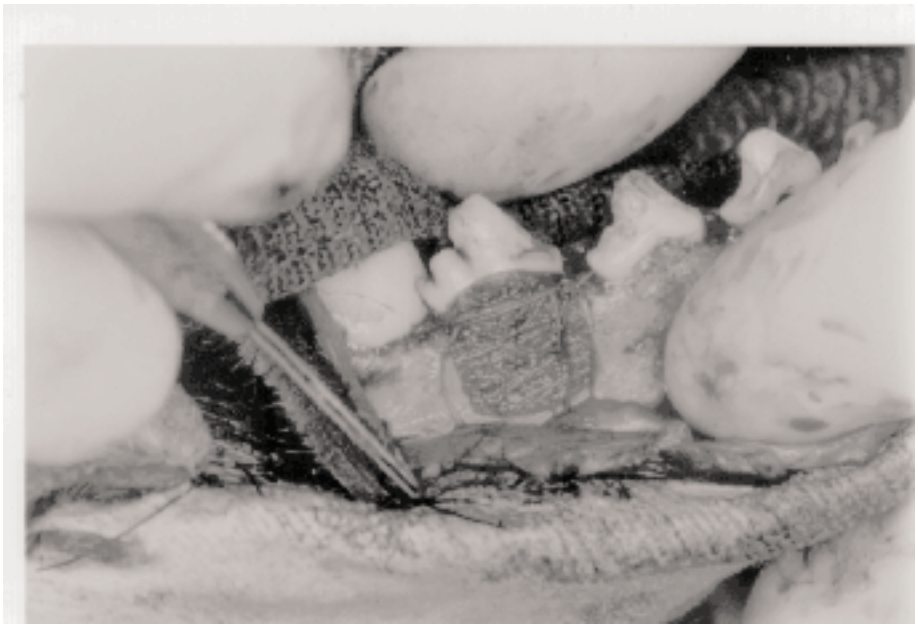
Results of this study indicate that healing of canine experimental grade III furcation defects is not enhanced by treatment with concentrated AF and/or RES barrier membrane compared with periodontal debridement and coronal flap repositioning based on analysis of histomorphometric parameters and digital subtraction radiography. Histopathologic assessment indicated that treatments including RES essentially prevented ankylosis and root resorption. Long-term clinical trials are recommended to assess the incidence and pathologic sequelae of ankylosis in veterinary dental practice when comparing periodontal debridement alone with resorbable barrier membrane application.

FIGURES

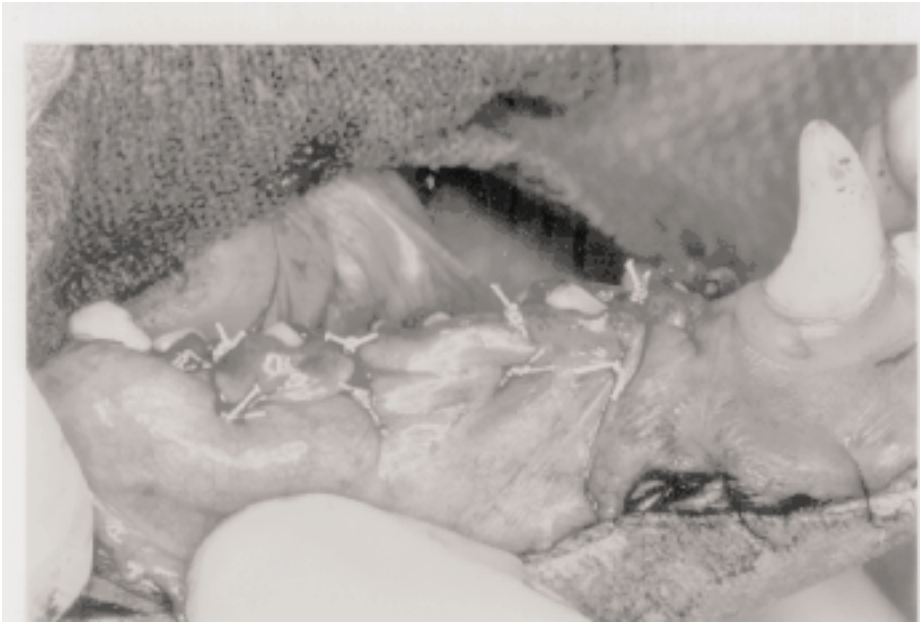


(Figure 1A)

(Figure 1B)



(Figure 1C)

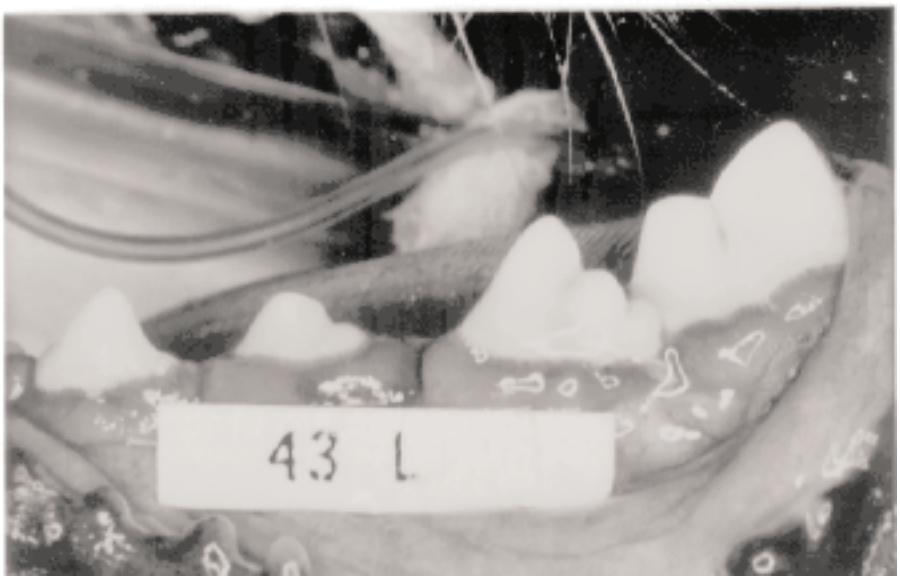


(Figure 1D)

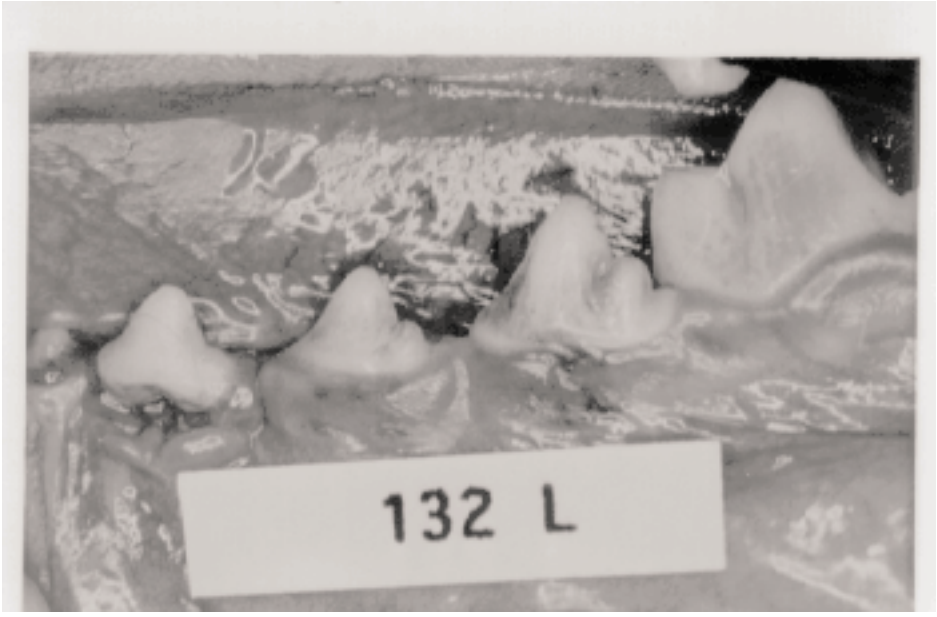
Figure 1 - Photograph of intraoperative view of AF application alone (A) and combined with RES (B). Note preplaced flap sutures. Final positioning of RES (C), and post-treatment view of the coronally repositioned flap (D).

(Figure 2)

Figure 2 - Photograph of ring bracket and intraoral film mount. Note aluminum reference wedge (Single arrow) on the film mount and polymethylmethacrylate impression material (Double arrow).



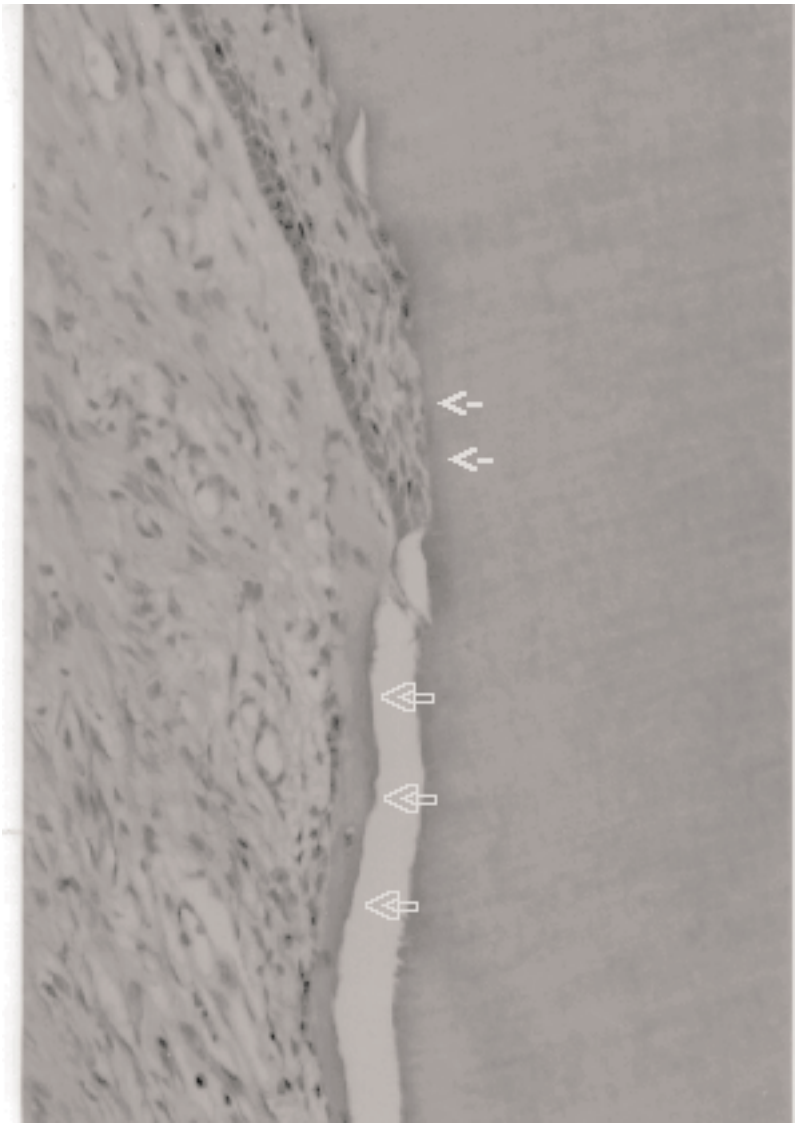
(Figure 3A)



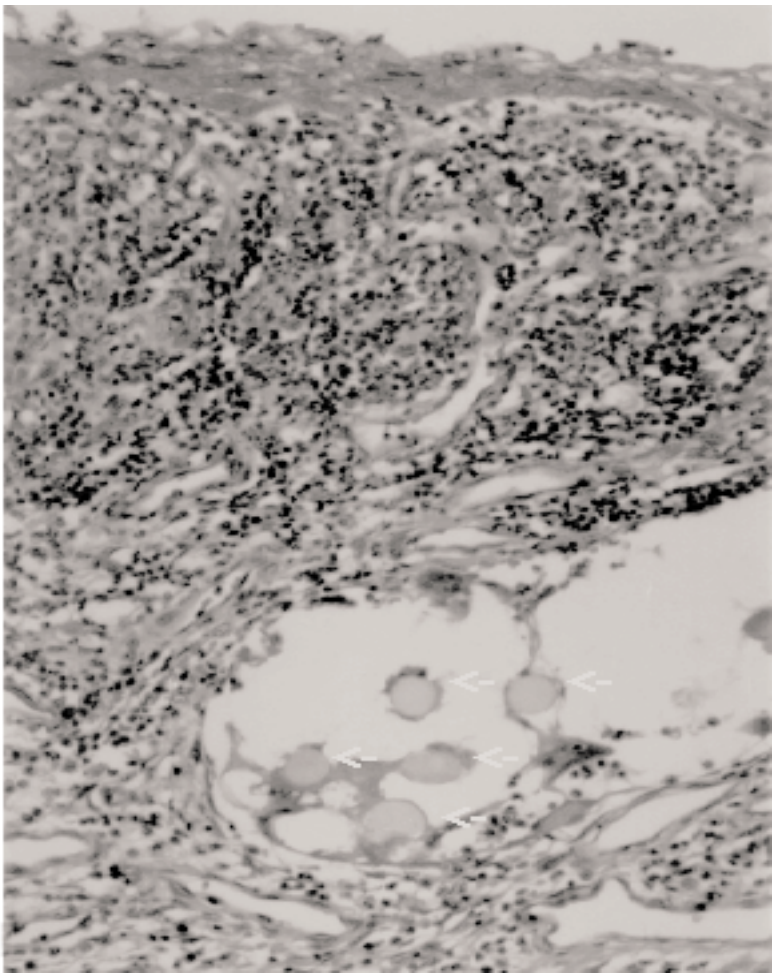
(Figure 3B)

Figure 3 - Photographs of typical gingival appearance at 3 (A) and 6 (B) months post-treatment. Flaps have receded from their immediate post-treatment position but remain coronal to the furcation entrance in 3 of 4 treated teeth.

(Figure 4A)

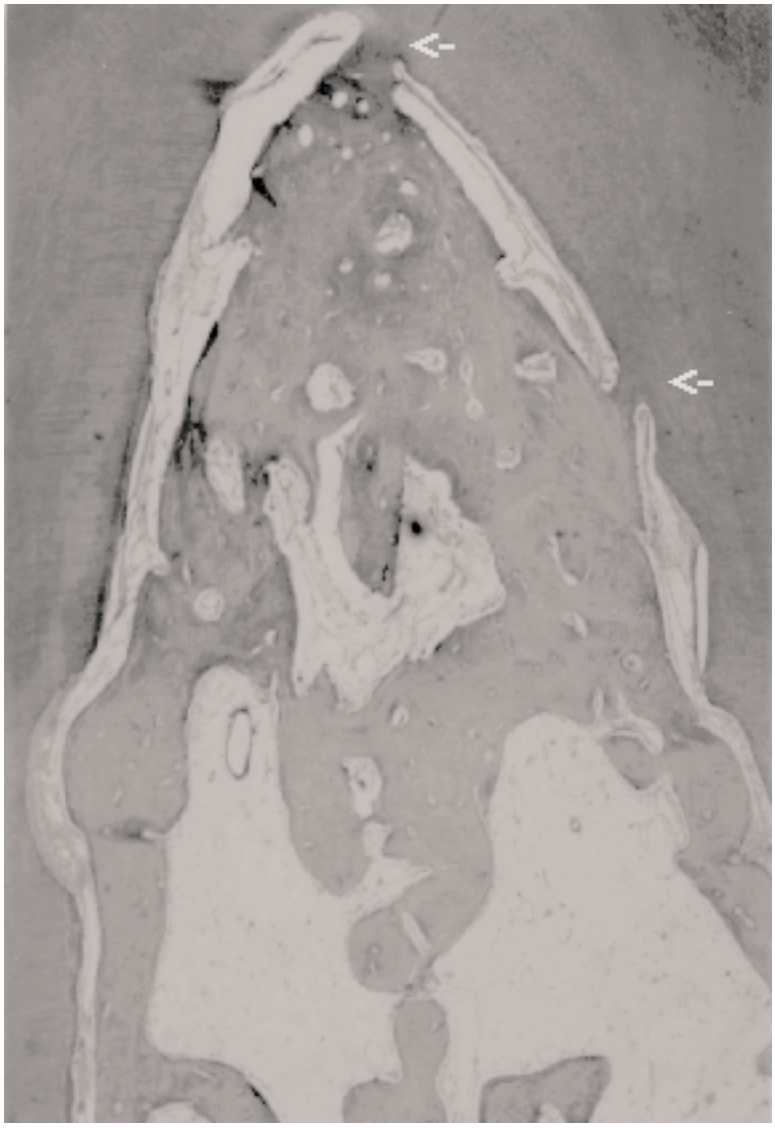


(Figure 4B)

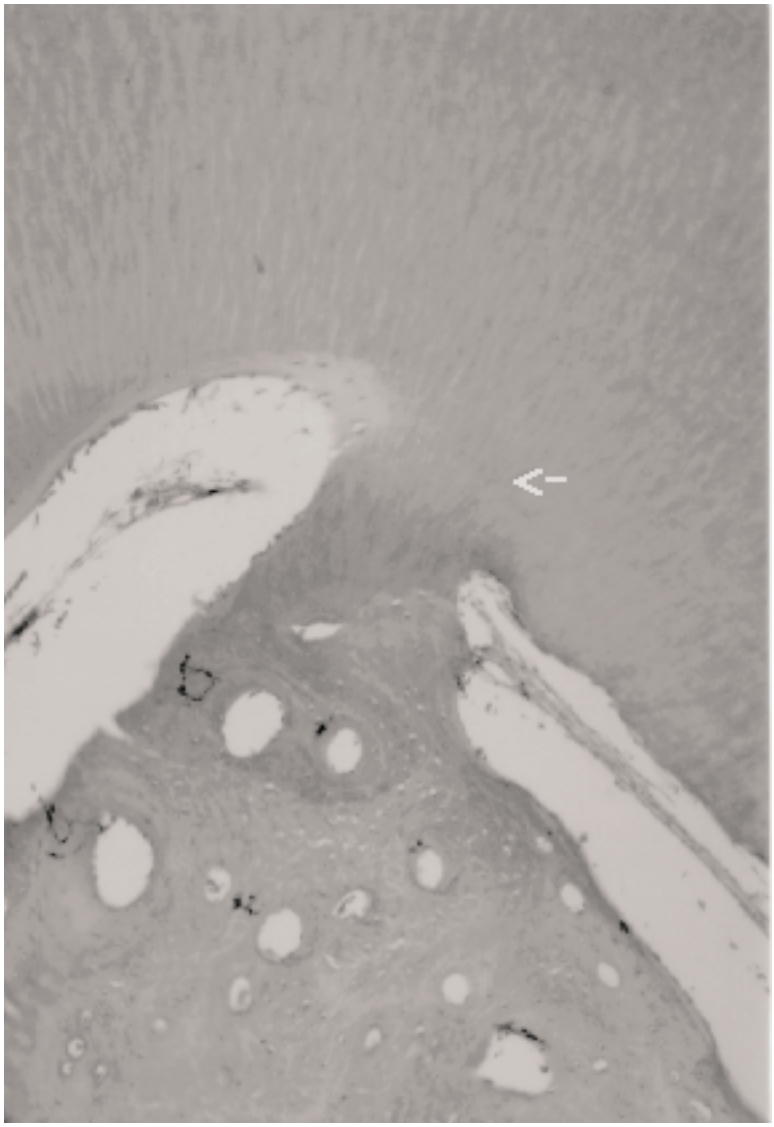


(Figure 4C)

Figure 4 - (A) Photomicrograph of a furcation defect 1 month following RES treatment. Measurement reference points include a radicular notch (solid arrow) and disrupted cementum and dentin (open arrow). A limited area of periodontal regeneration is present (small arrows) [5 x Magnification]. (B) Periodontal regeneration area juxtaposed with defect junctional epithelium (solid arrows). Note the immature, periodontal ligament-like tissue (PDL) and cementum (open arrows) which is artifactually separated from the dentin [66 x Magnification]. (C) The coronal aspect of the defect is composed of junctional epithelium, inflammatory cells, and immature connective tissue. RES fibers (arrows) are noted in the area of inflammation. [66 x Magnification]. Sections stained with hematoxylin and eosin.

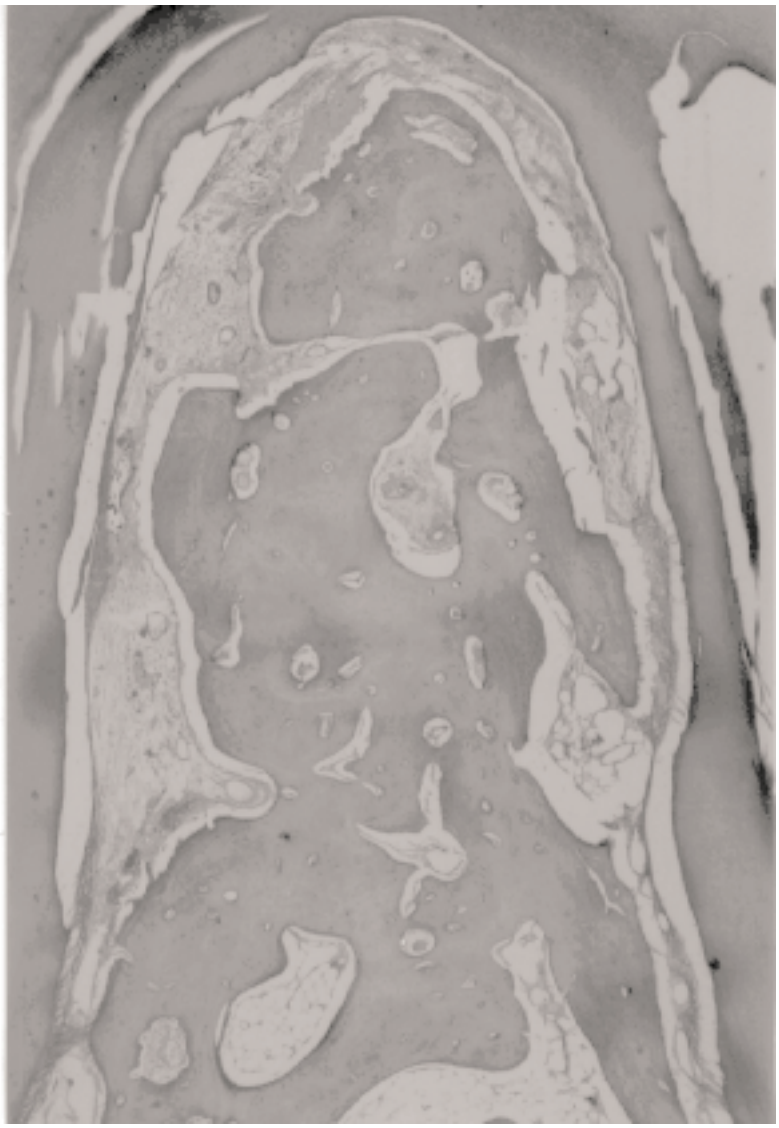


(Figure 5A)

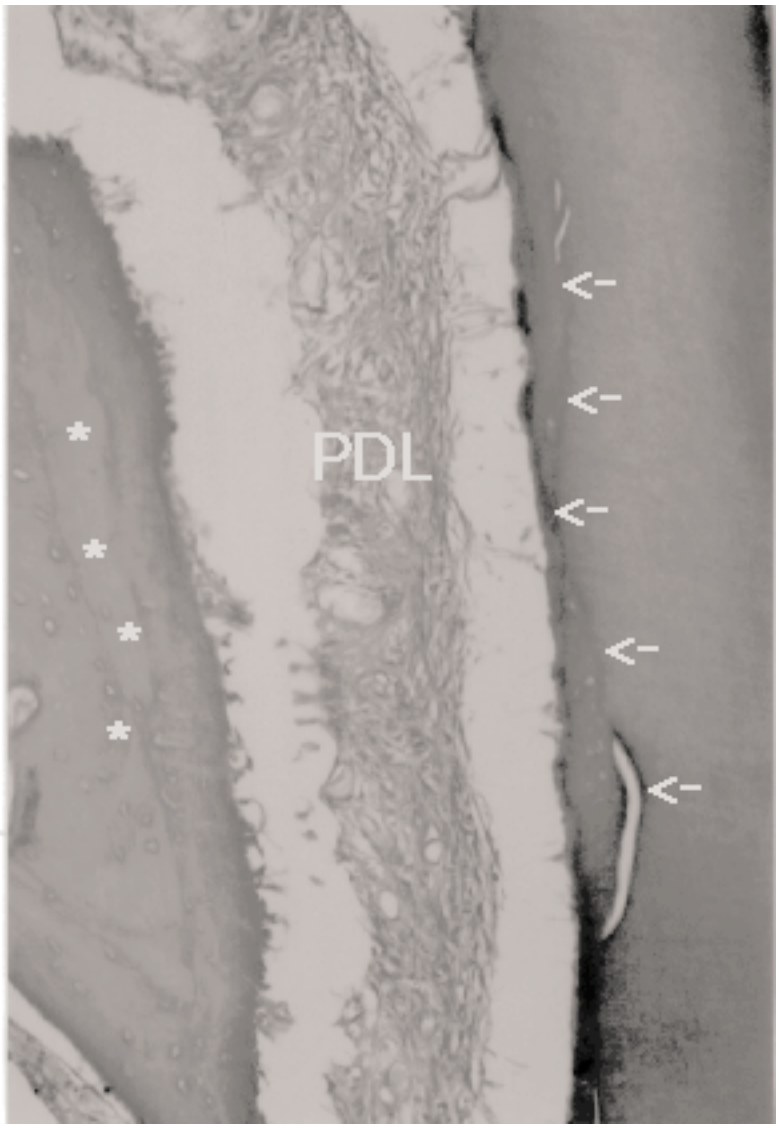


(Figure 5B)

Figure 5 - (A) Photomicrograph of a furcation defect 3 months following AF treatment. The defect is composed of new alveolar bone, periodontal ligament-like tissue, and cementum formation with small areas of root ankylosis (arrows) [6.6 x Magnification]. (B) Small area of root ankylosis (arrow) showing no associated resorption [25 x Magnification]. Sections stained with hematoxylin and eosin.

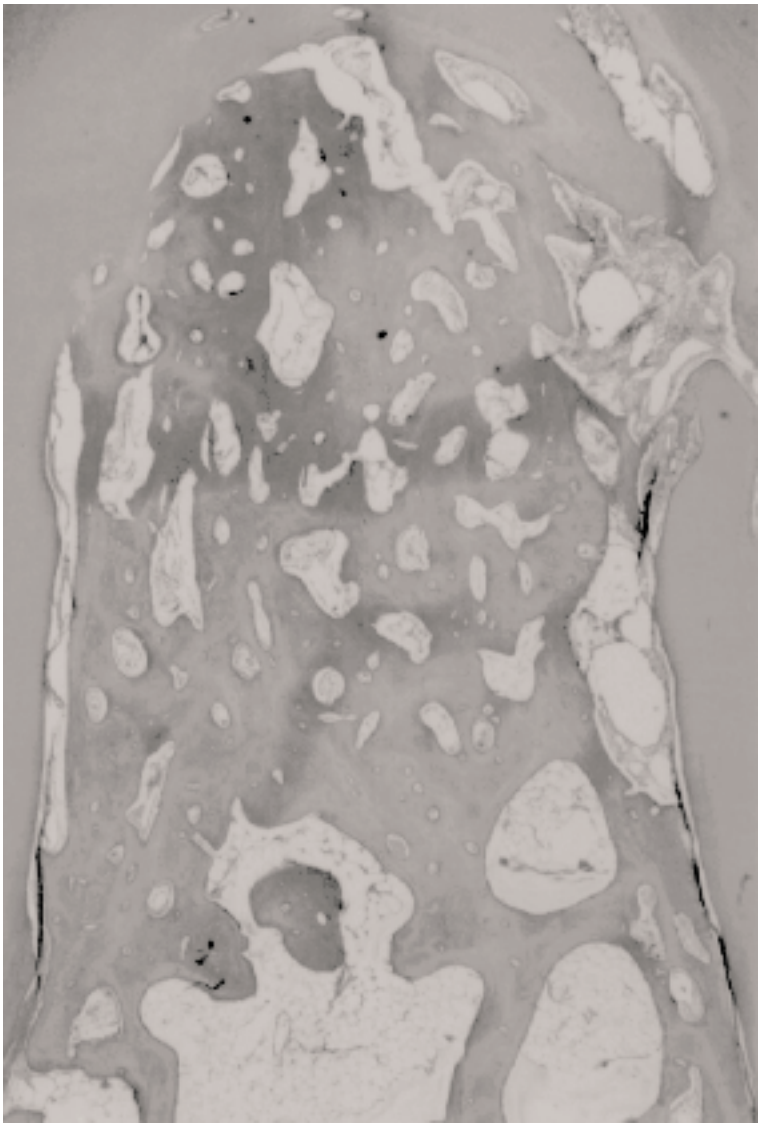


(Figure 6A)

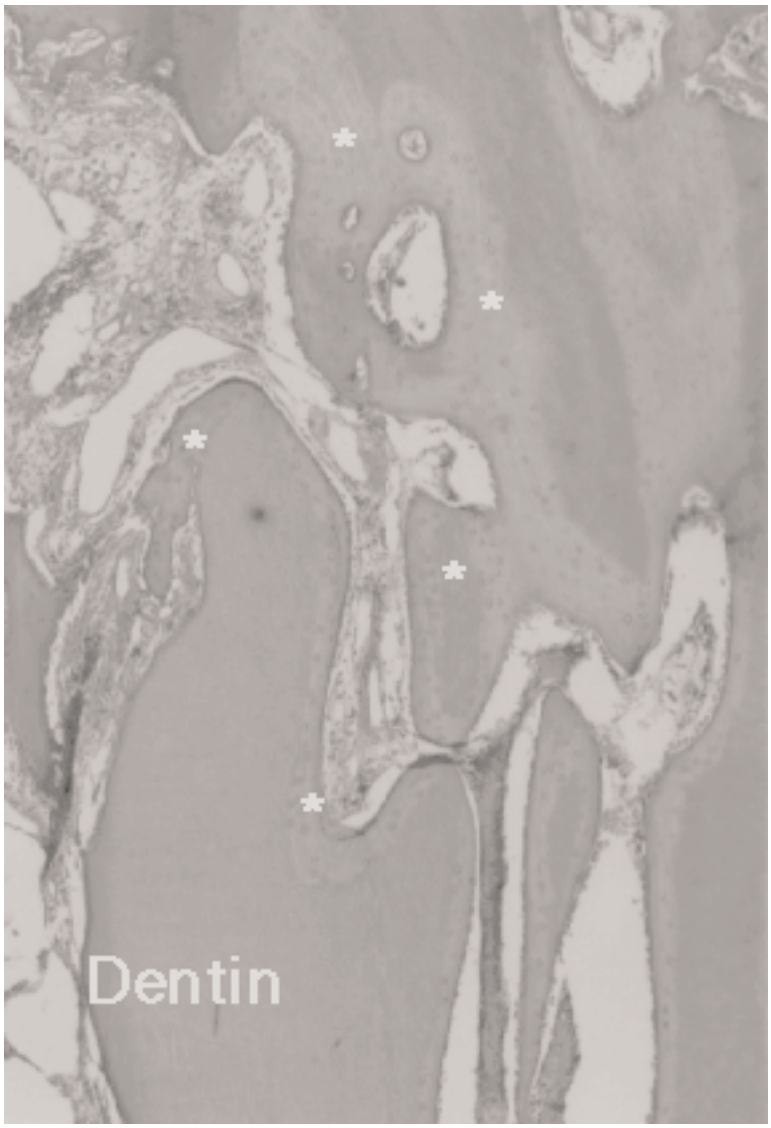


(Figure 6B)

Figure 6 - (A) Photomicrograph of a furcation defect 6 months following AF and RES treatment showing mature alveolar bone formation [6.6 x Magnification]. (B) Periodontal regeneration is noted by normalization of periodontal ligament-like tissue fiber orientation (PDL) between alveolar bone (*) and cementum (arrows) [40 x Magnification]. Sections stained with hematoxylin and eosin.



(Figure 7A)



(Figure 7B)

Figure 7 - (A) Photomicrograph of a furcation defect 3 months following AF treatment showing extensive ankylosis and associated root resorption at the coronal aspect of the furcation defect [6.6 x Magnification]. (B) Note areas of new bone formation (*) where dentin has been resorbed [16 x Magnification]. Sections stained with hematoxylin and eosin.

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Endnotes

[1]Impregum F, Espe, Seefeld, Germany.
[2]Vicryl, Ethicon, Somerville, NJ.
[3]Torbugesic 10 mg/mL, Ayerst-Montreal Laboratories, Ville St Laurent, Canada.
[4]Ketaset®, 100 mg/ml, Fort Dodge Laboratories, Fort Dodge, Iowa.
[5]PromAce, 10mg/ml, Aveco Co Inc, Fort Dodge, Iowa.
[6]CHX®, Vrx®, Products, Harbor City, Ca.
[7]Resolut® Regenerative material, Gore-Tex, W.L. Gore Associates, Inc., Flagstaff, Az.
[8]ePTFE; Periodontal material®; Gore-Tex, W.L. Gore Associates, Inc., Flagstaff, Az.
[9]Doxycycline Hyclate 100 mg. tablets, Zenith Goldline Pharmaceuticals, Inc., Ft. Lauderdale, Fl.
[10]Science Diet Canine Maintenance, Hill's Pet Products, Topeka, Ka.
[11]Beauthanasia-D Special, Pentobarbital sodium, 390 mg/mL, and Phenytoin Sodium, 50 mg/mL, Schering-Plough Animal Health Care Corp., Kenilworth, Nj.
[12]Technovit powder/liquid, Jorgensen Laboratories Inc., Loveland, Co.
[13]NIH - Image Ver. 1.61, National Institutes of Health, USA
[14]Minitab Statistical Software, Minitab Inc., State College, Pa.
[15]Dixon, BC. Evaluation of canine derived fibrin glue][Results of in-vitro analysis and use in full thickness skin grafts in dogs. 1995. MS Thesis. Department of Veterinary Sciences, University of Missouri, Columbia.

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VITA

Henri Chapman Bianucci was born on June 30, 1964 in Evanston, Illinois. He graduated from New Trier East High School in 1982 and then attended The University of Colorado at Boulder from 1982 thru 1985. At the University of Colorado he earned a B.S. in Business - Marketing. In 1986 he spent 6 months in a program of study on international organizations and French language through Kent State University in Geneva, Switzerland. Beginning in 1986 Henri began a position as general manager with Andens of Illinois, which was an import company involved in the importation of surgical textiles from mainland China. In 1988 Henri acquired a management position with ENM, Inc. of Chicago, which is the parent company to multiple original equipment manufacturers supplying companies such as Ford and Caterpillar. During his employment at ENM Henri took classes at Loyola University to fulfill his requirements for admission to veterinary school.

In 1990 Henri enrolled at the University of Illinois College of Veterinary Medicine and Graduated as Salutatorian in 1994. That same year he began a rotating internship in small animal medicine and surgery at the University of Missouri - Columbia. In 1995 he began his residency in small animal surgery and masters of veterinary sciences program at the Virginia-Maryland Regional College of Veterinary Medicine.

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