Periapical Healing following Endodontic Microsurgery with Collagen Based Bone Filling Material: A Randomized Controlled Clinical Trial

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Introduction: The purpose of this randomized controlled clinical trial was to evaluate two dimensionally and 3-dimensionally the effect of resorbable collagen-based bone filling material on periapical healing following endodontic microsurgery (EMS) on endodontic lesions presenting four-wall defect.

Methods: Thirty-nine cases with lesions of strictly endodontic origin and four-wall defect morphology underwent EMS and were randomly assigned to the treatment or control group. In the treatment group, a collagen-based bone filling augmentation material (Foundation, J. Morita USA) was placed into the osteotomy site after root resection, root-end filling, and induced bleeding. In the control group, the flap was repositioned after induced bleeding in the osteotomy with no material added. Clinical, PA, and CBCT examinations were completed after 12 months. Healing was evaluated using PA radiographs according to Molven's criteria and CBCT using PENN 3D criteria. Cortical plate healing was scored according to Chen's et al. and RAC/B index.

Results: Thirty-two cases were evaluated at the 12 months follow up consisting of 14 cases in the control group and 18 in the treatment group. A total of 11 cases in the control and 17 cases in the treatment group demonstrated complete healing on PA radiographs. On CBCT, 5 and 12 of cases had completely healed in the control and treatment groups, respectively. Finally, 13 cases in the treatment and 6 cases in the control group had reestablished cortical plate. However, none of the results were statistically significant.

Conclusion: Within the limitation of the present study, the use of collagen-based bone filling augmentation material did not show statistical significance in improving periapical healing when used as a coadjutant approach for endodontic lesions with the four-wall defect. Larger-scale studies are needed to provide more conclusive results.

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University of Pennsylvania Dental Medicine

Periapical Healing following Endodontic Microsurgery with Collagen Based Bone Filling Material: A Randomized Controlled Clinical Trial

THESIS

Amenah Albagle D.D.S
06/22/2021

Presented to the Faculty of Penn Dental Medicine in Fulfillment of the Requirements for the Degree of Master of Science in Oral Biology

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ACKNOWLEDGEMENT

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1) **Endodontic Microsurgery: Procedure, Wound Healing and Outcome**

Apical periodontitis is an inflammatory response of periapical tissues to bacterial infection within the root canal system resulting in tissue destruction (1). Endodontic treatment targets the causative agent to the inflammation by eliminating or substantially reducing the microbial load within the infected root canal (2,3). Despite the high success rate of primary root canal treatment (4–7), periapical lesions may persist or develop post-operatively in some instances. Endodontic retreatment to manage persistent periapical pathology can be done with nonsurgical retreatment or with a surgical approach. The treatment plan is a multifactorial decision; based on the patient’s medical history, type and quality of coronal restoration, quality of existing root filling, accessibility to root canals, patient’s preference, and the clinician’s skills and experience.

Surgical retreatment is generally recommended when obstruction to the apical part of the canal is anticipated. Cases with complicated anatomy resulting from extreme curvatures or calcifications, iatrogenic errors, or the presence of long posts can be treated predictably with endodontic surgery. In addition, management of a well-treated tooth with persistent apical periodontitis is preferably done through a surgical approach. Apical surgery in these situations can address intraradicular infection in the complex anatomy of the root canal system and possible extraradicular etiologies such as extraradicular infection (8), foreign body reaction (9), cholesterol crystals, and cysts (10).
Surgical Management of Apical Periodontitis:

Treatment is initiated by reflection of soft tissue flap to expose the underlying bone and root structure. Bone is then removed to gain access to the apex of the tooth and the inflammatory lesion. Curettage of the lesion is performed, and about three millimeters of the root apex is resected. This resection addresses more than 90% of apical ramifications within the root canal systems (11). Those anatomical structures present challenges during mechanical and chemical debridement in orthograde treatment (12). Next, root-end preparation is performed, where 3 millimeters of the intracanal root filling is removed. Finally, a filling material is added to the root-end preparation to seal and prevent ingress of intracanal irritants into the periapical tissues.

While the principles of endodontic surgery remain the same, the technical procedure has evolved remarkably over the years. Traditionally, surgery was performed with the naked eye or with loupes providing low magnification. It involved round burs in micro-handpiece for root-end preparation and amalgam filling as root-end filling. Advancements in the procedure include microsurgical instruments and incorporation of surgical microscope for magnification and illumination, ultrasonic tips for root-end preparation, more biocompatible root-end fillings (11). These developments have added precision, reproducibility and accuracy with a small surgical wound and hence made this procedure an established treatment option with a predictable outcome. A meta-analysis comparing the outcome of traditional root-end surgery with Endodontic Microsurgery (EMS) showed a statistically significant difference in success rates between the two techniques (59% vs. 94% respectively) (13).
Periapical Healing Following the Surgical Procedure:

The surgical procedure creates an excisional wound with a resected root surface and a bone defect within the alveolar process. Following the procedure, granulation tissues emanate from the severed PDL and endosteum and proliferate within the coagulum to regenerate lost tissues. The PDL-derived tissue is primarily responsible for dentoalveolar healing, and the endosteal-derived tissue is responsible for osseous healing (14). Therefore, periapical healing involves apical attachment regeneration over the resected root surface and osseous regeneration of trabecular and cortical bone within the defect (15).

Although this description compartmentalizes healing into three distinct areas (resected root, trabecular bone, and cortical plate), there is an interplay between the regenerated tissues that stimulate the formation of one another. Apposition of new woven bone begins within the internal surface of the wound and progresses externally (16). As the new bone contacts the delimiting membrane of the overlaying connective tissue, it induces its maturation into a functioning periosteum. The cortical plate is formed under the control of the functioning periosteum externally and the endosteal tissues internally. Similarly, contact of the endosteal tissue with the PDL-derived tissue over the resected root induces the encapsulating tissue to initiate osteogenic activity, thus developing a functioning periodontal ligament (14).

Regeneration and Repair:

The process of wound healing involves highly programmed phases of hemostasis, inflammation, proliferation, and remodeling (Guo) and can lead to regeneration or repair. Regeneration is the replacement of the damaged tissue by the same cells with the restoration of the biological function of the injured tissue. On the other hand, repair is the replacement of the damaged
tissue by a different one, such as fibrosis or scarring, and usually causes the loss of biological function of the injured tissue (17).

Regeneration of periapical tissues after periapical surgery requires (1) recruitment of progenitor/stem cells to differentiate into committed osteoblasts, PDL cells, and cementoblasts; (2) growth factors as necessary signals for attachment, migration, proliferation, and differentiation of progenitor/stem cells; and (3) local microenvironmental cues such as adhesion molecules, and extracellular matrix and associated noncollagenous protein molecules (18). Those elements must coordinate in time, space, and concentration to reconstitute damaged periapical tissues.

In small lesions, resident osteoblast, PDL cells, and cementoblasts can regenerate damaged tissues through cell division and proliferation. However, in large lesions, recruitment and differentiation of progenitor cells/stem cells are required. In the periapical wound, paravascular spaces of PDL contain adult stem cells that are capable of differentiating into PDL-like, cementoblast-like, and osteoblast-like cells. In addition, bone marrow mesenchymal stem cells and periosteal osteoprogenitor cells can differentiate into osteoblasts (18).

Like most postnatal wounds, the excisional wound following endodontic surgery cannot be completely regenerated (17). This is clearly demonstrated by the repair on the resected root where lost dentin cannot be regenerated. Furthermore, while the optimal outcome of regeneration includes restoration of the architecture of the damaged tissue, this aspect is not commonly evaluated in endodontic literature. The focus on regeneration in endodontic is mainly on the osseous fill of the surgical defect.
Using block biopsy, which included the root tip and the surrounded periapical soft tissue and bone, Andreasen and Rud studied healing following endodontic surgery (19). Histologically, three patterns of healing have been observed:

The first type is healing with the reformation of periodontal membrane or ankylosis. This is the most desirable outcome with regeneration of bone in the apical area. The second type is associated with varying degrees of periapical inflammation and involves repair with fibrous scar tissue in communication or adjacent to the periodontal membrane. The last mode involves the undesirable outcome of moderate or severe periapical inflammation without scar tissue.

Outcome Assessment:

Histological examination of the periapical tissues gives the most accurate information about healing following endodontic surgery. However, more clinically applicable methods involve a clinical examination and an evaluation of a follow-up radiograph.

The radiograph is evaluated for the resolution of the radiolucency signifying bone regeneration (20). The healing occurs along a continuum where the bone fill and its corresponding radiopacity on the radiograph gradually increase over time. Therefore, the radiolucency present on a follow-up radiograph could represent a stage in bone regeneration, fibrous scar tissue, or inflammatory changes due to inadequate treatment.

Using the histological findings described above, radiographic criteria were developed to assess the outcome following endodontic surgery (20). These criteria require an observation period of at least one year and classify healing using PA radiograph into four categories: complete healing, incomplete healing (scar tissue), uncertain healing, and unsatisfactory.
Radiographic Tools and limitations:

Traditionally and for decades, the outcome of endodontic surgery was measured using periapical (PA) films for healing evaluation (20). This tool presents a two-dimensional image of a three-dimensional object. The visualization of a lesion on a periapical radiograph depends on its location in different types of bone (9) and x-ray angulation (21). Lesions that are near or in the cortical bone are more readily visualized than those within the cancellous bone (22).

Furthermore, studies evaluating the radiographic interpretation process using both conventional and digital radiography have concluded that it is a subjective one (23–25), impacted by the evaluator’s years of experience, biases, and in the case of digital radiography, familiarity with the system (25).

In contrast to PA imaging, Cone-beam Computed Tomography (CBCT) provides an undistorted three-dimensional representation of the teeth and their surrounding structures (26). Therefore, this imaging modality overcomes the limitations of conventional 2D radiography, namely and most importantly, elimination of anatomical noise and geometric distortion.

Several studies have reported increased sensitivity of CBCT in the detection of periapical lesions compared to PA imaging (27–31). Because outcome assessment is a diagnostic process to evaluate periapical tissues post-treatment, CBCT’s higher sensitivity has implications for determining endodontic treatment success. Similar to nonsurgical treatment (32,33), studies evaluating the outcome of EMS showed that CBCT yielded a lower success rate than PA Radiographs (34–37).
Given the in an animal study, Chen et al. 2015 evaluated the outcome of EMS histologically and radiographically using different imaging modalities. The authors described different healing parameters to evaluate the outcome using CBCT. These included healing over the resected root surface, the periapical area, and the cortical plate. The present CBCT scoring criteria have incorporated the buccolingual dimension in determining the outcome (46), a dimension that was not previously accessible on PA radiographs. These parameters were later evaluated in a prospective clinical trial to determine radiographic healing one year following endodontic surgery (35). The results demonstrated that the suggested scoring criteria were repeatable, reliable, and applicable in human subjects. Adapted from the above-mentioned parameters, RAC/B indices (35) and Penn 3D criteria (37) have been suggested to interpret the outcome of EMS using CBCT. The RAC/B indices score healing on the resected root surface (R), apical area (A), and cortical plate (C). In addition, an overall score for bone healing (B) is provided. On the other hand, Penn 3D criteria classify healing into complete, limited or, unsatisfactory healing. The use of such criteria enhances intraobserver and interobserver agreement of healing interpretation as viewed on the CBCT (35). However, it has not been correlated with histology to determine its accuracy. It remains to be ascertained whether a residual low-density area on follow up CBCT is associated with inflammation and/or whether a longer follow-up period will lead to reduction in lesion size or be completely eliminated.
Bone Regenerative Techniques: Benefits and Materials

Bone Regenerative Techniques (RT) in Endodontic Surgery:

Deficiency in the bone tissue is considered one of the prognostic factors influencing endodontic surgery outcomes (38). Different parameters of the bony defect have been shown to affect healing, such as the size of the periapical lesion ((39,40), the width of the bony crypt (41), the height of the buccal bone plate (38), and the presence of apicomarginal defect (42).

Two main objectives have been described for applying regenerative techniques (RT) in endodontic surgery; to accelerate periapical healing and allow for best possible histological outcome in compromised clinical situations (43). A prospective clinical trial evaluating the outcome of endodontic microsurgery reported significantly lower success rates for lesions of combined endodontic-periodontal origins than strictly endodontic lesions (42). Healing in the combined lesion is often characterized by a long junctional epithelium along the denuded root surface with an increased risk of recurrent communication between marginal and apical tissues (43).

In large apical lesions, particularly those with defects in both facial and lingual cortical plate (through-through lesions), healing might occur with fibrous connective tissue core originating from the fast-growing soft tissues. Since the scar tissue presents as a radiolucent area on a radiograph, this form of healing described as incomplete healing creates a diagnostic challenge for the clinicians with a risk of overtreatment.

The use of regenerative techniques in endodontic surgery has been evaluated by several studies (54–61) with substantial heterogeneity regarding study design, surgical procedure,
lesion type, applied regenerative material, and outcome evaluation method (44,45). There is a consensus within the endodontic literature that RT has beneficial effects on periapical healing of teeth presenting with apicomarginal defect (43–45). This conclusion is based primarily on animal studies (46–48) and uncontrolled clinical studies (42,49,50).

For lesions of strictly endodontic origin, several randomized controlled clinical trials have demonstrated that the use of RT enhanced periapical healing of through and through defects (51–53). In one RCT, the success rate of through and through defects treated with the combined use of resorbable collagen membrane and anorganic bovine bone was significantly better than control cases (88% vs. 57% respectively) (53).

In contrast, variable results have been reported from studies evaluating strictly endodontic lesions with four-wall defects (Table 1). In 1995, Pecora showed that large circumscribed periapical lesions healed more rapidly when e-PTFE membranes (Gore-Tex Periodontal Material (W.L. Gore & Associates, Flagstaff, AZ) covered the defect (51). In another RCT, complete regeneration of bone was observed clinically, radiographically, and histologically in sites receiving a combination of bone graft and membrane barrier (54). Other studies have shown no added benefits of using regenerative techniques to heal osseous defects limited to the periapical area. Garrett et al. evaluated the rate of periapical healing of osseous defect following endodontic surgery with or without the use of Guidor (Guidor, Besenville, IL) bioresorbable membrane. Densitometric image analysis of digital imaging has shown no statistical difference between osseous healing rates in the two groups (55). In another study, the use of PerioGlas (US Biomaterials Corporation, Florida, USA), a bioactive glass bone substitute, did not significantly improve endodontic healing outcomes in the long term (56).
Finally, Tascheiri et al. 2007 showed that the use of inorganic bovine bone mineral (Bio-Oss, Geistlich Biomaterials, USA) and resorbable collagen membrane (Bio-Guide, Geistlich Biomaterials, USA) had no beneficial effect on the outcome of periapical surgery of strictly endodontic lesions (57).

Based on the studies mentioned above, several published reviews have concluded that there is no significant advantage for regenerative techniques in strictly endodontic apical lesions with four-wall defects (45,57,58). The studies have several limitations, including limited sample size and the use of radiopaque bone graft, which hinders the interpretation of radiographic healing. Moreover, none of the mentioned studies have evaluated the healing following endodontic surgery with RT using CBCT. Studies evaluating endodontic microsurgery with CBCT in strictly endodontic lesions have reported lower success rates than 2D imaging (35–37). More importantly, CBCT evaluation revealed patterns of healing with deficiencies in the cortical plate formation. A long-term study evaluating outcome using CBCT demonstrated that cortical plate reestablishment was challenging to achieve, with only 42% of cases showing complete healing at the five-year follow-up (59). The incorporation of CBCT compounds the need for reevaluation of regenerative techniques as a prognostic factor in healing assessment.

Collagen-Based Bone Filling Material:

Different strategies have been developed in the periodontic field to reconstitute lost alveolar bone, such as guided bone regeneration (GBR) with occlusive membranes and bone grafting techniques. The techniques allow bone healing through cell occlusion of fast-growing gingival connective tissue, space provision of the defect, and blood clot stabilization (60).
Moreover, local application of growth factors, cytokines, and host modulating agents are being used to promote bone regeneration (61).

Among regenerative techniques, bone grafting materials are widely to promote new bone formation at defect sites. The material can be osteogenic, providing osteocompetent cells that begin the bone-forming process. Osteoinductive grafts release growth factors or mediators that stimulate the host mesenchymal stem cells to differentiate and begin bone formation. Finally, a graft that provides scaffolding for the host to create new bone and has no biological influence on the host is osteoconductive (62).

Due to some inherent problems associated with grafting materials such as infection, limited supply, and graft rejection, there is a growing interest in alternative bone graft materials such as metals, polymers, and ceramics to fill the bone defect (63). Collagen, a natural polymer, is the most abundant animal protein providing structural and mechanical support to tissues and organs (64). It has many favorable features, including biocompatibility, low antigenicity, and biodegradability, allowing it to be used as a biomaterial and tissue engineering scaffold (65).

Foundation (J. MORITA USA) is a collagen-based bone filling augmentation material for use after teeth extraction. It is made from atelocollagen, which promotes new bone formation by stimulating osteoblast differentiation and endogenous collagen production (66). More specifically, two types of atelocollagen in Foundation are crossed linked to form the material. Fibrillar collagen, providing scaffolding for surrounding cells, and Heat Denatured Atelocollagen stimulate cells' infiltration into the scaffold (67). In an animal study where Foundation was placed in a freshly extracted socket, woven bone was well developed, and alveolar bone
volume was maintained at four weeks following extraction. In contrast, control cases demonstrated bone volume resorption during the same evaluation period (68). Histological evaluation of human extraction sockets showed active woven bone formation eight weeks after the use of Foundation (J. MORITA USA) (69).

The material has merits in its ease of use and application, structural stability negating the need for membrane, and most importantly, its radiolucent nature which allows masked radiographic evaluation of healing following endodontic surgery. Although several studies evaluated the material's efficacy in post-extraction sites in animal and clinical studies (67,70–72), no previous study has evaluated its effect on the healing of periapical lesions.
Table 1. Studies evaluating regenerative techniques (RT) in endodontic surgery with four-wall defects.

<table>
<thead>
<tr>
<th>Author and Year</th>
<th>Type of Study</th>
<th>Number of Subjects</th>
<th>Lesion Size</th>
<th>Follow Up</th>
<th>Regenerative Material</th>
<th>Evaluation Method</th>
<th>Outcome</th>
<th>Comments</th>
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<tr>
<td>Pecora et al. 1995</td>
<td>Randomized Clinical Trial</td>
<td>Test= 10 Control= 10</td>
<td>≥10 mm</td>
<td>3, 6, 9 and 12 months</td>
<td>e-PTFE membranes (Gortex)</td>
<td>Clinical and Radiographic (PA)</td>
<td>Radiographic complete healing: 6 m: Control= 20% Test= 80% 12 m: Control=90% Test= 90%</td>
<td>Results are combined from through-through lesions and four-wall defects. Study limitations: Small sample size. Healing criteria not described. Randomization method not described.</td>
</tr>
<tr>
<td>Tobon et al. 2002</td>
<td>Randomized Clinical Trial</td>
<td>Test 1= 10 Test 2= 10 Control= 10</td>
<td>0.04–506 mm² (radiographic)</td>
<td>12 months</td>
<td>Test 1= e-PTFE membranes (Gortex) Test 2= resorbable hydroxyapatite (OsteoGen®)+ e-PTFE membranes (Gortex)</td>
<td>Clinical, Radiographic and Histological</td>
<td>Radiographic complete healing: Control= 44% Test 1= 66% Test 2= 100% Histological: Control= 25% bone, 25% scar, 50% granuloma Test 1= 62.5% bone, 12.5% scar, 25% granuloma Test 2= 100% bone</td>
<td>Study limitation: Small sample size. Randomization method not described. Radiopacity of material could interfere with radiographic interpretation.</td>
</tr>
<tr>
<td>Garrett et al. 2002</td>
<td>Randomized Clinical Trial</td>
<td>Test= 9 Control=4</td>
<td>-</td>
<td>3,6, 9 and 12 months</td>
<td>Polylactide bioreabsorbable membrane (Guidor®)</td>
<td>Densiometric analysis of PA radiographs</td>
<td>No significant difference in densiometric ratio between groups (p=0.6133)</td>
<td>Study limitations: Small sample size. Large drop-out rate (48%)</td>
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<tr>
<td>Taschieri et al. 2007</td>
<td>Randomized Clinical Trial</td>
<td>Test= 16 Control= 22</td>
<td>≥10 mm</td>
<td>12 months</td>
<td>Inorganic bovine bone mineral (Bio-Oss®)+ and collagen membrane (Bio-Gide®)</td>
<td>Clinical and Radiographic (PA)</td>
<td>Radiographic complete healing: Control= 82% Test= 88%</td>
<td>Study included through-through lesions. Result shown from four-wall defects only. Study limitation: Small sample size. Radiopacity of material</td>
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<tr>
<td>Study</td>
<td>Period</td>
<td>Group 1</td>
<td>Group 2</td>
<td>Short term</td>
<td>Long term</td>
<td>Treatment</td>
<td>Clinical and Radiographic Healing</td>
<td>Radiographic Healing</td>
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<tr>
<td>Pantchev et al. 2009</td>
<td>Retrospective</td>
<td>110</td>
<td>76</td>
<td>&gt;5 mm</td>
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<td>Bioactive glass (PerioGlas®) in Group 2 only</td>
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<td>Short term: 9 m to 2 years</td>
<td>Long term: 33 m to 4 years</td>
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<td>Dominiak 2009</td>
<td>Prospective Clinical Study</td>
<td>26</td>
<td>30</td>
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<td>Mean of 9 mm</td>
<td>6 and 12 months</td>
<td>Test 1: resorbable collagen membranes (Bio-Gide®)</td>
<td>Test 2: xenogenic Bio-Oss Collagen material®</td>
<td>Test 3: xenogenic Bio-Oss Collagen® material in combination with platelet rich plasma</td>
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<td>Clinical and Radiographic (PA)</td>
<td>Radiographic complete healing: Short term: Group 1 = 54% Group 2 = 72% (p&lt;0.05) Long term: Group 1: 84% Group 2 = 74% (p&gt;0.05)</td>
<td>Study limitations: - Low level evidence - Radiopacity of material could interfere with radiographic interpretation.</td>
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- Low level evidence
- Radiopacity of material could interfere with radiographic interpretation.
References


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Study Objectives

The aims of the study are:

1. To evaluate the effect of resorbable collagen-based bone filling material on periapical healing following Endodontic Microsurgery (EMS) with clinical and radiographic examinations using Periapical radiographs (PA) and Cone Beam CT (CBCT) scan after 1 year.

2. To identify prognostic factors that influence healing following EMS as observed on CBCT.

The primary null hypothesis is:

*There is not a statistically significant difference in periapical healing of EMS using resorbable collagen-based bone filling material vs control.*

The secondary null hypothesis is:

*There is not a statistically significant difference between any prognostic factor and periapical healing following EMS.*
Abstract:

Introduction: The purpose of this randomized controlled clinical trial was to evaluate two dimensionally and 3-dimensionally the effect of resorbable collagen-based bone filling material on periapical healing following endodontic microsurgery (EMS) on endodontic lesions presenting four-wall defect.

Methods: Thirty-nine cases with lesions of strictly endodontic origin and four-wall defect morphology underwent EMS and were randomly assigned to the treatment or control group. In the treatment group, collagen-based bone filling augmentation material (Foundation, J. Morita USA) was placed into the osteotomy site after root resection, root-end filling, and induced bleeding. In the control group, flap was repositioned after induced bleeding in the osteotomy with no material added. Clinical, PA and CBCT examinations were completed after 12 months. Healing was evaluated using PA radiographs according to Molven’s criteria and CBCT using PENN 3D criteria. Cortical plate healing was scored according to Chen’s et al. and RAC/B index.

Results: Thirty-two cases were evaluated at the 12 months follow up consisting of 14 cases in the control group and 18 in the treatment group. A total of 11 cases in the control and 17 cases in treatment group demonstrated complete healing on PA radiographs. On CBCT, 5 and 12 of cases had completely healed in the control and treatment group, respectively. Finally, 13 cases in the treatment and 6 cases in the control group had reestablished cortical plate. However, none of the results were statistically significant.

Conclusion: Within the limitation of the present study, the use of collagen-based bone filling augmentation material did not show statistical significance in improving periapical healing when
used as a coadjutant approach for endodontic lesions with four-wall defect. Larger-scale studies are needed to provide more conclusive results.

**Introduction**

Persistent periapical pathology in root canal treated teeth can be managed through endodontic microsurgery. The current procedure employs high magnification and illumination, micro-instruments, ultrasonic root-end preparation, and more biocompatible root-end fillings (1). These developments added precision that made this procedure a well-accepted treatment option with a predictable outcome. Studies evaluating endodontic microsurgery have demonstrated a high success rate in the short and long term (2,3).

In addition to the resolution of clinical signs and symptoms, the regeneration of lost tissues due to pathological and procedural processes is an essential determinant of healing following surgery. Several studies have evaluated the use of bone regenerative techniques such as membrane, grafts, or both, to enhance new bone formation following endodontic surgery (4–10). Bone regenerative techniques have been demonstrated to improve periapical healing of through-through lesions (5,9) and apicomarginal defects (11–13). The results of different studies evaluating healing of strictly endodontic lesions with intact lingual plate (four-wall defects) are more controversial. Pecora et al. reported that large circumscribed periapical lesions healed more rapidly using the membrane technique (4). In 2002, Tobon et al. reported enhanced clinical, radiographic, and histological healing using regenerative techniques compared to the control, where defects were allowed to heal spontaneously (7).

Other studies have shown no added benefits of using regenerative techniques for osseous defects limited to the periapical area. Garrett et al. 2002 evaluated the rate of periapical healing
of osseous defect following endodontic surgery with or without the use of Guidor (Guidor, Besenville, IL) bioresorbable membrane (6). Densitometric image analysis of digital imaging has shown no statistical difference between osseous healing rates in the two groups. Tascheiri et al. 2007 showed that the use of inorganic bovine bone mineral (Bio-Oss, Geistlich Biomaterials, USA) and resorbable collagen membrane (Bio-Guide, Geistlich Biomaterials, USA) had no beneficial effect on the outcome of periapical surgery (8).

The radiographic evaluation in the above-mentioned studies is based on conventional periapical imaging. The incorporation of Cone Beam Computed Tomography (CBCT) in outcome assessment allows undistorted, three-dimensional visualization of teeth and surrounding tissues. In particular, the buccolingual dimension allows evaluation of cortical plate, a parameter that was not previously evaluated on periapical imaging (14). Studies evaluating healing following endodontic microsurgery with CBCT reported a lower success rate compared to 2D imaging (15–18). Furthermore, observation of healing patterns using CBCT reveals incomplete bone regeneration well beyond the one-year follow-up (19).

Considering the inconsistent results of previous studies regarding the efficacy of bone regenerative techniques in endodontic surgery and in light of the observed pattern of healing on CBCT, a re-evaluation of bone regenerative techniques as a prognostic factor is warranted. The purpose of this randomized controlled clinical trial was to evaluate periapical healing of strictly endodontic lesions with four-wall defects following endodontic microsurgery with collagen-based bone filling augmentation material, Foundation (J. Morita USA) using 2D and 3D imaging.
Materials and Methods

This study was a randomized, evaluator blinded, and controlled clinical trial. The research protocol was approved by the ethics committee of Institutional Review Board of the University of Pennsylvania (IRB Protocol #: 833538). The study is registered on clinicaltrials.gov under ID NCT0451499. The surgical and outcome evaluation procedures used for this trial followed guidelines previously reported by Safi et al. 2019(17).

Sample Size Determination, Power and Enrollment:

The minimum sample size was determined to be 74, based on 30% mean difference in outcome between the groups, power = 0.80 (P < .05) and 20% assumed dropout rate. Subjects were recruited from Sept 2019 to December 2020.

Inclusion Criteria:

1) Patients 18 years or older with a noncontributory medical history and no contraindication for oral surgery.

2) A history of previous endodontic treatment with persistent apical periodontitis.

3) True endodontic lesions with Kim and Kratchman’s classification: A, B, or C (Table 1)(1).

4) Intact coronal restoration without sign of leakage.

Exclusion Criteria:

1) Minors and patients with contraindications for oral surgery.

2) Lesions with Kim and Kratchman’s classifications: D, E, and F (Table 1) (1).

3) Through-through lesions.

4) Single lesion involving multiple teeth.
5) Insufficient coronal restorations.

6) Vertical root fractures.

After evaluation for eligibility criteria, patients scheduled for undergoing endodontic microsurgery procedure at the University of Pennsylvania Endodontic Department were invited to participate. Eligible individuals were enrolled in this study. Subjects were fully informed about the study, and possible risks and benefits involved with the study procedures. Written informed consent forms were obtained.

**Preoperative Procedures:**

Preoperative data were collected and included: subject’s sex, age, tooth involved, diagnosis, clinical symptoms, lesion classification, treatment rendered before EMS (primary non-surgical root canal treatment, nonsurgical retreatment, previous history of apical surgery). A PA radiograph (DEXIS™ Platinum Sensor; KaVo Dental, Brea, CA) and a limited FOV CBCT scan were taken. The preoperative CBCT was acquired using Veraviewepocs 3D R100 (Morita, Irvine, CA): FOV = 4 x 4 cm, voxel size = 0.125 mm, and it was assessed for the following preoperative factors:

1) Presence of cortical plate fenestration. This information was verified clinically during surgery.

2) Thickness of the buccal bone: measured 3 mm coronally from the apex on the B-L view.

3) Lesion size: height, depth and width of the lesion were measured. The value of the largest dimension was recorded as the lesion size.

4) Root angulation: measured between the long axis of the root and a line tangent to the cortical plate at the resection area.
**Surgical Procedure:**

The surgical procedures were performed by calibrated postgraduate endodontic residents under the supervision of faculty members. For all cases, routine EMS protocol was performed under operating microscope according to Kim and Kratchman’s guidelines(1). Research participants used (Peridex - Chlorhexidine Gluconate 0.12%) mouthwash for two days prior to the surgery. In addition, participants were premedicated with an NSAID (400 mg Etodolac/600 mg ibuprofen) one hour before procedure. Participant’s medical history was reviewed, and the surgical area was locally anesthetized with 2% lidocaine 1:50,000 epinephrine. An additional mandibular block with 2% lidocaine 1:100,000 epinephrine was administered for mandibular molars. Full-thickness flap was reflected, and a 3 mm root-end resection with 0-10° bevel was done using Lindemann bur. The resected root surface was dried with Stropko irrigator, stained with methylene blue, and inspected under high magnification (x16- x25). Ultrasonic tips were used for root-end preparation, and root-end was filled with Endosequence Root Repair Material (RRM; Brasseler, Savannah, GA) . Using a periodontal probe, the final dimensions (depth x width x length) of the osteotomy were recorded. The osteotomy site was then curetted to induce bleeding.

**Randomization:**

Randomization was performed before flap repositioning using a publicly available software tool (Randomizer for Clinical Trials©). At each randomization assignment, the patient code was entered, and the application randomly assigned the treatment arm to the osteotomy site (A/Foundation, B/Control). In multirooted teeth with separate osteotomies, each root was randomized independently.
The test group received EMS plus foundation collagen-based bone filling augmentation material (J. MORITA USA) placed into the osteotomy and the control group received standard of care EMS alone. For the test group, before placement of Foundation, the material was trimmed using a sterile instrument to a size slightly larger than the defect to ensure direct contact with the bone. The Foundation material was allowed to infiltrate with the induced blood and once moist, it was manipulated to be flush with the buccal bone. In the control group, the flap was reapproximated, and sutures placed without any material added to the site. For all cases, Nylon sutures were placed for primary wound closure and post-operative PA radiograph (DEXIS™ Platinum Sensor; KaVo Dental, Brea, CA) were taken. Post-operative instructions given to all participants, and they were prescribed with oral analgesic (600 mg of ibuprofen or 400 mg of Etodalac). Sutures were removed 3-5 days post-operatively.

Follow Up Procedures

Subjects were invited to attend to a follow-up study visit after one year of the surgical procedure, for clinical and radiographic examination. Teeth were assessed for reported symptoms, sensitivity to palpation and percussion, mobility, and probing depths. The presence of intraoral swelling or sinus tract was recorded. PA radiograph (DEXIS™ Platinum Sensor; KaVo Dental, Brea, CA) of the involved teeth were obtained using paralleling technique with XCP holders (Denstply Rinn, Charlotte, NC). Limited 3D-volume CBCT scan (Veraviewepocs 3D R100; Morita, Irvine CA) was obtained at 90 kV and 8 mA.
Outcome Assessment:

Three calibrated evaluators assessed the radiographs independently, blinded to the treatment arm. Cases with disagreement between the evaluators were discussed until a joint agreement was reached. Radiographs and CBCT scans were viewed in a dark room and projected on a big screen. Preoperative, post-operative, and follow-up PA radiographs were first compared, and healing was determined using Molven’s criteria (20) (Figure 1) as complete, incomplete, uncertain and unsatisfactory healing. Following the assessment by PA, CBCT scans were reviewed by the same evaluators using One Volume Viewer (J Morita MFG. Corp, Kyoto, Japan) in the multiplanar reconstruction mode and with high-definition projection. The sectioning planes were adjusted for proper alignment before dynamic evaluation of the full volume CBCT scan. The sagittal plane was aligned parallel to the M-D plane and the coronal plane was aligned with the longitudinal axis of the tooth. The slice thickness was set to 0.125 mm. Evaluators were allowed to reformat CBCT images for better assessment. Healing was determined based on Penn 3-dimensional criteria described by Safi et al. (17) (Figure 2). Cases were classified into complete, limited, or unsatisfactory healing. The outcome was dichotomized into success (Complete and incomplete: 2D, Complete and limited: 3D) and failure (uncertain and unsatisfactory: 2D, unsatisfactory: 3D). Teeth presenting with symptoms at the follow-up appointment were regarded as failure irrespective of the radiographic presentation. In addition, the evaluators determined cortical plate healing using the scoring system described by Chen et al. (14) and Von arx et al. (19).
Cortical Plate Healing Scores:

Score 0: Cortical plate not re-established.

Score 1: Cortical plate is partially re-established.

Score 2: Cortical plate is re-established.

Statistical Analysis:

Fisher’s exact test was used to evaluate significant association between different healing categories and prognostic factors. Exact logistic regression models were performed to assess odds of complete healing as a linear combination of prognostic factors. The osteotomy site was considered as the unit of analysis. A probability of $P < .05$ was assigned as the level of significance.

Table 1. Microsurgical Classification of Periapical Lesion

<table>
<thead>
<tr>
<th>Class A</th>
<th>Absence of periapical lesion, with normal pocket depth and no mobility. Unresolved clinical symptoms after nonsurgical approach.</th>
</tr>
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<tbody>
<tr>
<td>Class B</td>
<td>Small periapical lesion with normal pocket depth and no mobility.</td>
</tr>
<tr>
<td>Class C</td>
<td>Large periapical lesion with normal pocket depth and no mobility.</td>
</tr>
<tr>
<td>Class D</td>
<td>Large periapical lesion with no mobility. Deep noncommunicating pocket depth present.</td>
</tr>
<tr>
<td>Class E</td>
<td>Periapical lesion with endodontic-periodontal communication to the apex. No obvious fracture present.</td>
</tr>
<tr>
<td>Class F</td>
<td>Periapical lesion and complete denudement of the buccal plate without mobility.</td>
</tr>
</tbody>
</table>
Figure 1. Molven’s Criteria. (a) Complete Healing Category. When the lamina dura is restored to original width (A). When the lamina dura is reconstituted but is less than two times the width along the resected root surface (B). When the lamina dura is widened along the root end filling material (C). Complete bone repair, but the density of bone in the surgical site is not the same as the surrounding bone (D). No discernible lamina dura or pdl at the resected root surface, suggesting ankylosis. (b) Incomplete healing category (scar tissue). The radiolucent area at follow-up has decreased but there is a dense radiolucency that is asymmetric to the apex and has a dense compact border often with a sun burst bone pattern (A). A dense radiolucent area not in continuity with the pdl within the surgical site (B). (c) Uncertain healing category. Here (A) represents the radiolucency as seen on an immediate postoperative radiograph and (B) represents the follow-up. The area has reduced significantly but is still larger than two times the original pdl space. (d) Unsatisfactory healing category where (A) represents the radiolucency as seen on an immediate postoperative radiograph and (B) represents the follow-up. The area has enlarged in size or remains the same. Reprinted from Microsurgery in Endodontics (p.214), By B.Karabucak. M. Kholi, F. Setzer, 2017, John Wiley & Sons, Incorporated
Figure 2. Penn 3D criteria for success. (a) Complete healing category. (A) Reformation of periodontal space of normal width and lamina dura over the entire resected and unresected root surfaces. (B) Slight increase in the width of apical periodontal space over the resected root surface, but less than twice the width of non-involved parts of the root. (C) Small defect in the lamina dura surrounding the root end filling. (D) Complete bone repair with discernible lamina dura; bone bordering the apical area does not have the same density as the surrounding non-involved bone. (E) Complete bone repair with hard tissue covering the resected root end surface completely. No apical periodontal space can be discerned. (b) Limited healing category. (A) The radiolucent area at follow-up has decreased significantly but the continuity of the cortical plate is interrupted by an area of lower density. (B) Bone repair in the surgical site but a low-density area remains asymmetrically located around the apex or has an angular connection with the periodontal space. (C) Significant bone repair has taken place but the bone has not fully formed in the area of the former access osteotomy. (D) The cortical plate has completely healed but there is a low-density area near the resected root surface. (c) Unsatisfactory healing category. The volume of the low density area appears enlarged or unchanged. Reprinted from Microsurgery in Endodontics (p.214), By B.Karabucak. M. Kholi, F. Setzer, 2017, John Wiley & Sons, Incorporated
A total number of 67 subjects were recruited from September 2019 to December 2020.

The patient pool included 72 teeth with 86 roots treated and randomized independently. Patients were consecutively enrolled until March 10th, 2020, after which all clinical and research activity was suspended due to the COVID-19 pandemic. The study was resumed in August 2020. The preliminary analysis described herein consist of results from 31 patients recruited from the September 2019 to March 2020 (Figure 3). Follow-up time ranged from 11-17 months with a
mean of 12 months. The 31 patients represented 35 teeth (40 roots) with 39 osteotomy sites randomized. Of those, 24 patients representing 32 osteotomy sites presented for the one-year follow-up (77% follow up rate). A total 14 cases in the control group and 18 in the treatment group were fully evaluated. The demographic distribution of cases is presented in Table 2.

Two cases, one in each group, presented with symptoms, and vertical root fracture in both teeth were confirmed during exploratory surgery. These two cases were not included in the radiographic analysis. A total of 13 cases in the control group and 17 cases in the Foundation group were analyzed radiographically using PA radiographs and CBCT scans.

The overall success rate on both imaging modalities, was 90.6% with 85.7% success rate for the control group and 94.4% for the treatment group. Similar results were obtained on both PA radiograph and CBCT with respect to the outcome. The difference in outcome between groups when assessed on both PA and CBCT, was not statistically significant. Table 3 provides the distribution of cases in each healing category as evaluated on PA radiographs and CBCT scans. On the PA radiographs, 11/14 cases in the control and 17/18 cases in the treatment group showed complete healing after one-year post-surgery. On CBCT, 12/18 cases in the treatment group showed complete healing on CBCT compared to 5/14 in the control group. The cortical plate was completely re-established (C-Score =2) in 13/18 cases of the treatment group and in 6/14 of the control group. The results were not statistically significant. Figure 4 shows examples of the healing observed on the one-year CBCT scan. Serial models of exact regression analysis by treatment and each covariant (Table 2) showed no statistically significant influence of on complete healing in all evaluated factors.
<table>
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<td>16-31 degree</td>
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### Table 3. Distribution of Radiographic Healing in Asymptomatic Cases.

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<th>INCOMPLETE</th>
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<tr>
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**Figure 4.** Healing on CBCT of Control (A-B and E-F) and Foundation (C-D and G-H) cases. A) Preoperative B-L section of #10. B) 1 year follow up scan showing limited healing with the continuity of cortical plate interrupted by area of low density. C) Preoperative B-L section of #7. D) 1 year follow up scan showing complete healing. E) Preoperative B-L section of #14. F) 1 year follow up scan showing limited healing with incomplete bone formation in area of former osteotomy site. G) Preoperative B-L section of #3. H) 1 year follow up scan showing complete healing.
**Discussion:**

Several clinical studies have investigated the use of different regenerative techniques to enhance bone healing following endodontic surgery (4–6,8–10,22,23). However, these studies vary in design, surgical procedure (traditional vs. microsurgery), lesion type, regenerative materials, evaluation method, and outcome criteria. These variations hinder accurate and direct comparison of results between studies.

Considering lesion type, clinical studies evaluating radiographic healing of four-wall defects with regenerative techniques used conventional PA radiograph as an imaging modality, which has limitations affecting its diagnostic accuracy. Although our study used CBCT in addition to PA for assessing healing outcome, which has been demonstrated to be a more sensitive imaging approach, our results agree with previous studies showing no added benefit to regenerative techniques in the healing of periapical lesions with four-wall defects on PA radiographs (6,8).

Three-dimensional criteria on CBCT incorporated cortical plate healing in the assessment of outcome following endodontic microsurgery (14,17,25). A recent study comparing healing five years following apical surgery reported advanced osseous healing of the defect along the resected root surface and in the apical area. Yet only 42% of the cases had complete reestablishment of cortical plate (19) at five years post-surgery. This study demonstrated that teeth undergoing EMS take longer than one year to heal when no regenerative material is used.

CBCT evaluation in the present study revealed to be a better imaging modality to evaluate the influence of the regenerative material, and although a higher difference between
groups in the complete healing category was found when evaluators assessed the images by CBCT; the difference was not statistically significant. Data showed that 66% of cases in the Foundation group had completely healed compared to 35% in the control group. In addition, 72% of Foundation cases had complete cortical plate formation compared to 42% in the control group at the 12 months follow-up. While this could suggest a clinical benefit to the use of Foundation, we believe a larger sample size would have provided a stronger evidence.

Previous studies have shown that assessment of outcome using CBCT yields lower healing than PA radiographs (15–17,26). This was exemplified in our study by the fact that when evaluating healing categories, the proportion of complete healing was lower when assessed on CBCT (overall complete healing= 53%) than PA radiographs (overall complete healing= 87.5%). These results are in agreement with past research showing about 30% disagreement in ratings between PA and CBCT (18,30).

Different parameters of bone tissue in the surgical area were evaluated in this study as potential prognostic factors influencing periapical healing. None of the prognostic factors significantly impacted the healing when assessed by CBCT (data not shown). Among the evaluated factors are the presence of cortical plate fenestration (p=0.4713), the lesion size (p=0.1261), and microsurgical classification (p=0.6732). The results agree with Safi et al. study, which showed no significant impact of these factors on the outcome when evaluated by CBCT (17).

Collagen-based biomaterials have been widely used in tissue engineering due to their favorable properties such as biocompatibility, low antigenicity, and biodegradability(24). The material used in this study is a collagen-based bone filling augmentation material, Foundation
(J. MORITA USA). It is produced by cross-linking fibrillar (FC) and heat denatured atelocollagen (HAC) and is available in bullet shape molds. The FC+HAC composite has been described to stimulate migration of undifferentiated mesenchymal cells and provide a scaffold for bone regeneration (25). Moreover, atelocollagen promotes bone regeneration through the production of Type I collagen extracellular matrix (26) which plays a major role in osteoblastic differentiation (27). A major drawback of Foundation is its fast degradation time (2-4 weeks) (25,28), compromising its space maintenance function (28). Ideally, degradation rate of the scaffold should match the remodeling rate of the target tissue (29). To support bone formation and maturation, degradation within 5-6 months is considered appropriate (29,30). Although several studies evaluated the efficacy of the material in post-extraction sites in animal and clinical studies(25,31,32). No previous study has evaluated its effect on the healing of periapical lesions.

The results of our study should be interpreted with caution as the present sample provided inadequate statistical power. Due to the small sample size, which was a limitation of our trial, the data offers inconclusive evidence regarding the effect of collagen-based bone filling material on the periapical healing of four-wall defects following EMS. It remains questionable whether the complete sample would provide a similar difference between groups once analyzed and whether the difference would be statistically significant.

Large-scale studies are needed to provide more robust support and more conclusive evidence. Furthermore, different regenerative materials have been shown to have varying impacts on endodontic surgery outcome(33). Thus, evaluation of healing using different types of materials would be beneficial. Lastly, healing assessment at additional follow-up periods
would shed light on the healing dynamic and provide information on the cost-effectiveness of material in the long term.

**Conclusion:**
Within the limitation of the present study, the use of collagen-based bone filling augmentation material did not show statistical significance in improving periapical healing when used as a coadjuvant approach for endodontic lesions with four-wall defect. Larger-scale studies are needed to provide more conclusive results.

**Addendum:**

Follow up procedures of the total participants is expected to be completed in Dec 2021.

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