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Recent Advances in Clinical Trials

Outcome of Endodontic Microsurgery Using Resorbable Membranes and Lyophilized Human Bone Graft: A Prospective Randomized Controlled Clinical Trial

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ABSTRACT

Purpose: *The aim of this prospective randomized controlled clinical study was to evaluate the effectiveness of a resorbable membrane with and without the complement of human lyophilized bone grafts used as aids in endodontic microsurgery. The study was conducted in patients that presented with periapical pathologies that had not responded to primary endodontic treatment and retreatment.*

Methods: *From a cohort of 189 consecutive patients presenting with non-resolving periapical radiolucencies located at the maxillary anterior teeth with a size that covered at least two apices, 174 (92%) of them met the inclusion criteria. They were randomly allocated into three modalities of treatment ie. Group 1: conventional enucleation procedures (CENP; n=58). Group 2: enucleation with placement of resorbable Fascia Lata Membrane (FLM; n=58). Group 3: enucleation with placement of resorbable Fascia Lata membrane combined with lyophilized human bone graft (FLM-HBG; n=58). After enucleation the tissues were submitted for histopathological analysis. The surgical outcome was determined clinically and radiographically after 24 months postoperatively using standardized periapical radiographs and Cone Beam Computed Tomography (CBCT) imaging. Data was subjected to statistical analysis.*

Results: *Of the initial 174 treated patients, 120 responded to the 24-month recall. In Group 1, 21 cases out of 39 (54%) had successfully healed. Patients in Group 2 showed a success rate of 93% in39 cases out of 42 while patients in Group 3 achieved a success rate of 95% in 37 cases out of 39. The histopathological analysis showed that the most frequent lesions were periapical granulomas. Groups 2 and 3 showed significant differences with Group 1 ($P<0.001$), however, no significant difference was found between Group 2 and 3 ($P>0.05$).*

Conclusions: *The use of Fascia Lata resorbable membranes, whether with or without the addition of a lyophilized human bone graft, showed significantly better bone repair than conventional surgical procedures.*

Keywords
Bone healing, Fascia Lata membrane, Lophilized bone graft, Periapical surgery, Resorbable membranes.

Introduction
Periapical radiolucencies in the maxilla or mandible generally occur because of tumors, trauma or after surgical procedures due to the presence of periapical pathological lesions [1]. Repair of bone defects in the jaws is a complex process and incomplete healing may occur due to interference during the healing process [2]. Since the regeneration of bone tissue is much slower than that of the oral mucosa one of the main concerns in the healing of periapical lesions is the ingrowth of connective and epithelial tissues from the peripheral areas into the osseous defect after enucleation of the pathologic tissues [1,2]. These events cause anatomic disturbances which often required a surgical re-entry. Based on this evidence, complete healing of these lesions has been reported to be lower than 25% [2]. To address this problem, various techniques and biomaterials have been introduced. Different studies have been reported on the advantage of using guided bone regeneration procedures (GBR) with the aid of bio resorbable or non resorbable biologic membranes, either alone or in combination with bone grafts, aiding the successful outcome of the surgical endodontic treatment of these lesions [3-7]. The process of bone regeneration progresses from the periphery of the surgically generated cavity in a centripetal direction, while gradually reducing the bone defect. Thus, collagen membranes are thought to play a regulatory role in osteoblast cell differentiation and growth [8]. Among the various approaches to avoid connective tissue ingrowth after enucleation, the use of Fascia Lata membrane (FLM) which is a strong fascial layer and lyophilized human bone graft (HBG) were both recommended for different surgical situations [9,10]. FLM is a section of the thigh´s aponeurosis and a thick resistant membrane with great elasticity and flexibility. Presently it is widely used in the fields of medicine and dentistry [9,10]. In medicine FLM are used for treatment of urinary incontinence [11] and to repair abdominal wall defects or diaphragmatic alterations [12]. In dentistry, FLM have been used for experimental GBR in laboratory animals [13] and in clinical endodontic surgery in humans [14]. FLM are also used for ridge augmentation and alveolus preservation [9]. In addition, HBG is considered an ideal biomaterial to regenerate large bone defects due to its excellent osteoconductive properties [10]. In clinical endodontics, periapical radiolucent areas are usually assessed by periapical (PA) radiographs, cone-beam computed tomography (CBCT) [15] or by preoperative non-invasive biopsies [16].

Objectives
The aim of the present prospective randomized controlled trial was to investigate the outcome of endodontic microsurgery using resorbable FLM or a combination of FLM-HBG in comparison with conventional enucleation procedures (CENP) on the healing of human periapical lesions. The null hypothesis postulated that there are no significant differences in the outcome of the treatments performed with CENP or with the aid of FLM or FLM-HBG.

Methods
Study Design and Ethics
Patient Enrollment and inclusion/exclusion criteria
Between Jannuary 2011 and October 2014, 189 (n=189) consecutive patients presenting with periapical radiolucent areas on preoperative PA radiographs of maxillary anterior teeth were recruited from the Department of Endodontics at the University Dental Hospital, Faculty of Dentistry, University of Buenos Aires.

The inclusion criteria were defined as follows:
1. Patients over 18 years of age with non-contributory medical history who signed an informed consent form with respect to the surgical protocol and agreeing to preoperative, immediate postoperative and follow-up periapical radiographs (PA) and at least one follow-up CBCT evaluation at the end of the study (24 months).
2. Patients presenting radiographic evidence of persisting periapical lesions that had not responded to primary endodontic treatment and retreatment.
3. Patients presenting normal periodontal probing depth, no mobility, teeth located in the anterior area of the maxilla (ie: single rooted teeth) and lesions whose sizes involved at least two apexes.

The exclusion criteria were considered as follows:
1. Non consenting patients.
2. Women with advanced pregnancy or breast feeding, patients with diabetes or smokers.
3. Teeth with fissures, fractures, perforations or root resorption.
4. Traumatized teeth or non restorability of the coronal portion.

Randomization Procedures
From the initial cohort of 189 individuals, 174 (92%) of them met the inclusion criteria. All these patients were equally assigned to three groups of 58 individuals each using a robust zone group randomization procedure [17]. In Group 1, (the control group) patients were treated with CENP while in Groups 2 and 3 the patients were treated with FLM or a combination of FLM-HBG respectively.

The study was performed according to the 2013 revision of the Helsinki declaration of 1975. The study protocol was approved by the ethics committee of the institutional review board of the Faculty of Dentistry, University of Buenos Aires (Protocol FOUBA #3011-2011-8). The clinical trial was registered with Resolution (ID) 921-14 EXP-UBA911/20720130200007BA. The following data of the patients was collected: Age, gender, previous radiological studies, tooth location, presence or absence of a post, personal and family history and histopathology of the curetted tissues from the lesions. For reducing radiation exposure, preoperative, immediate postoperative and follow-up periapical radiographs (PA) or limited CBCT were obtained from the affected teeth under strict maintenance of the ALARA principles [18]. The present trial was reported according to the Preferred Reporting Items for Randomized Trials in Endodontics (PRIRATE) 2020 guidelines [19]. These items are shown in Figure 1.

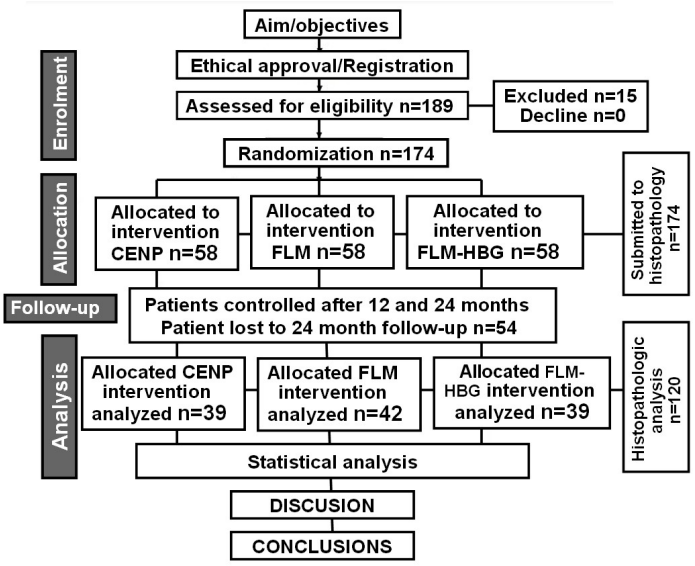

Figure 1: PRIRATE flowchart. Study design and flowchart of participants.

Surgical Procedures

The surgery was performed by one operator (PR) adhering to the same standardized protocol and with the aid of an OPMI PICO operating optical microscope (Karl Zeiss, Oberkochen, Germany). After injection of local anesthesia with 1.7 ml carticaine 4% L-adrenaline 1:100.000 (Bernabó Laboratories SA, Buenos Aires, Argentina) an incision was made followed by either a trapezoidal or triangular full mucoperiosteal flap. After curettage of the internal part of the flaps, osteotomy of the bone overlying the root apexes was performed with a piezoelectric scalpel tip in a piezoelectric unit (NSK, Osaka, Japan) under constant irrigation with sterile saline. The affected tissues were exposed and gently removed with microsurgical curettes. In case a cyst was diagnosed, care was taken to completely remove the lesion along with its outer capsule. After thorough curettage of the area, the removed tissues were placed in 10% neutral buffered formalin and submitted for histopathologic evaluation. Bone resection was limited to the absolute minimum to create the necessary space to do the curettage of the periapical area. Tetracycline powder dissolved in saline (two applications of 20 seconds each) flooded the bone cavity followed by thorough washing with saline. The root tips were ultrasonically sectioned to a length no less than 3 mm measured from the anatomical apex using the piezoelectric unit and a diamond tip, taking care that the remainder of the root was adjusted to be perpendicular to the long axis of the tooth. Hemostasis was achieved by applying pressure with sterile gauze soaked in adrenaline. The resected root surfaces were stained with 1% methylene blue dye and inspected under X20 and X25 magnifications to examine the cleanliness and the presence of cracks or fissures. A retrograde preparation of 3-4 mm depth was carried out with the piezoelectric unit at low power setting using a E4D ultrasonic tip (NSK) and sterile water coolant. Retrograde back filling of the root canal was then performed with progressive increments of EndoSequence BC RRM fast setting putty material (Brasseler, Savannah, GA, USA). The material was compacted manually with micro filling pluggers (Hu-Friedy Mfg. Co. Chicago). After the bone cavities were thoroughly rinsed with saline, patients were treated according to the above mentioned modality of treatment.

Bone Cavity Treatment

In Group 1 (control), the bone cavities were treated with the CENP. In Group 2, the bone cavities were treated with the aid of FLM (Bioxen, Biotecnologies SRL, Santa Fe, Argentina). FLM was soaked in saline until it obtained the desired consistency and then placed on the cortex of the bone cavity in such a way that it was 3 mm above the contour of the bone cavity. In Group 3 the bone cavities were filled with a combination of FLM-HBG. HBG was used in the form of particulate bone powder (Bioxen) that had been applied to FLM. In all groups, the flaps were repositioned, and the wounds closed with silk sutures making sure that the flaps were not under tension. The patients were instructed to treat the surgical area with Chlorhexidine Digluconate 0.12% gel every 3 or 4 hours for the first 3 days and then 3 or 4 times per day for 7 days. Antibiotics were prescribed starting one day before surgery and for 5 days postoperatively, Amoxicillin 1g every 12 h. In addition, Ibuprofen 400 mg every 8 hours for 4 days was recommended when necessary. The sutures were removed 5-7 days postoperatively.

Follow-up clinical, radiographic and CBCT evaluation

The patients were contacted for follow-up examination by telephone and/or e-mail. They were controlled clinically and radiographically immediately after surgery and after 12 and 24 months postoperatively using parallel digital PA radiographs (Carestream DENTAL RVG 5200, Atlanta, GA). Criteria for clinical success were based on the absence of clinical manifestations such as tenderness to percussion, spontaneous pain or pain during palpation and no changes such as fistula, swelling or periodontal pocket formation. The case was considered a failure when pain, fistula or inflammation was present. PA radiographs were digitalized and transferred to a computer. Two calibrated and blinded experienced Endodontists (PR and OZ), evaluated independently the images using Image J 1.38x Image Analysis Software (National Institutes of Health, Bethesda, MD) in a 400 x 350 mm screen with high quality contrast in a dark room. PA radiographs were evaluated according to the Molven´s et al. criteria [20]. Success (complete healing) was considered when the radiolucent area had totally disappeared, and the bone had formed around the root without interruptions. If the radiolucency had decreased in size by at least 50% and the patient was free of clinical signs and symptoms, the case was considered as incomplete healing or healing in progress. In such cases the patients were advised that continued monitoring was necessary since the new control will be an important part of the study. When the radiolucency had not changed or increased over time, the case was considered as failure (nonhealed).

Each patient had two limited-volume CBCT scans (CBCT 9000 3DC Carestream FOV 2,5 x 5, France) with patient consent: one preoperative and one at the 24-month follow-up. CBCT images

were also projected in a dark room with a slice thickness of 0.130 mm. After the images were aligned, healing was evaluated according to the modified Penn-3 criteria described by Schloss et al. [21]. The results were dichotomized into two categories: healed and nonhealed. Cases categorized as complete healing or healing in progress on CBCT evaluation in absence of clinical signs and/or symptoms were considered as success. Cases categorized as nonhealed with or without clinical manifestations were considered as failure. Patients who are symptomatic at the 24-month follow-up were considered as failures irrespectively of PA or CBCT features. In cases of PA and CBCT evaluation for which disagreement occurred between the examiners, the questioned case was discussed until agreement. The Interexaminer and intra examiner agreement was calculated using the Cohen´s Kappa coefficient.

Statistical Analysis

Data was entered into a database for statistical analysis using the IBM SPSS statistical package 20.0 software (IBM SPSS Inc., Chicago; IL, USA) setting at a range of admissible values for the dependent and the independent variables. To describe categorical data, absolute and relative frequencies with 95% confidence intervals were calculated. The relationship between categorical variables was evaluated by analysis of variance (ANOVA), the Snedecor (F) test and the Mantel-Haenszel x2 test. Bonferroni´s correction test was used to evaluate whether in one group the success/failure outcome of the treatment was significantly different from the others. The significance level was set at $P<0.05$.

Results

The Kappa scores ratio was k=0.89 for interexaminer agreement and k=0.68 for intra examiner agreement. From the 174 treated patients, 169 (97.12%) were able to come to the 12-month recall. Almost all of them sowed incomplete healing except for two cases of Group 2 and one case of Group 3 who presented a status of healing in progress. The final results of success (healed) and failure (nonhealed) rates were determined using the data recorded at the 24-month recall. The recall rate was 69%. From the initially treated cohort of 174 individuals, 120 of them ie, 39, 42 and 39 from subsequently Groups 1, 2 and 3 were available for the final follow-up evaluation. All data collected from the 120 patients who presented at 24-month recall visit were entered in a computer program. The patients' median age, range and gender are shown in Table 1. The percentages of success and failure rates according to the type of treatment (groups) and the corresponding 95% confidence intervals are shown in Table 2 and 3 respectively. After 24 months, 21 cases out of 39 (53.84%), 39 cases out of 42 (92.85%) and 37 cases out of 39 (94.87%) were considered successful for Groups 1, 2 and 3 respectively as observed on PA radiographs and CBCT scans (Figures 2, 3 and 4). There were no significant differences between PA and CBCT observations ($P>0.05$). Age, gender, tooth location, radiolucency size, clinical signs and symptoms, presence of previous root canal treatment and/or retreatment failures, histopathologic examinations of periapical lesions and the presence or absence of intrarradicular posts had no significant effect on the outcome of the surgical treatment ($P>0,001$). There was a significant association between the type of treatment (groups) and the success of bone repair. Patients treated with FLM or FLM-HBG showed significant differences with those treated with CENP ($P<0.001$). No significant differences between Groups 2 and 3 were found ($P>0.05$). Based on the results, the null hipothesis was partially rejected.

Table 1: Distribution and range of patients according to age and gender.

Gender	n	Age	Median (SD)	%
Women	57	18 – 63	38.24 ± 1.32	47.5
Men	63	18 – 52	34.53 ± 1.17	52.5

Table 2: Absolute frequency and percentage of success after 24 months.

	n	AF	%	CI
Group 1	39	21	53.84	39 – 68
Group 2	42	39	92.85	81 – 98
Group 3	39	37	94.87	83 – 99

AF: Absolute frequency; CI: 95% Confidence interval (lower and upper limits).

Table 3: Absolute frequency and percentage of failure after 24 months.

	n	AF	%	CI
Group 1	39	18	46.15	32 – 61
Group 2	42	3	7.14	2 – 19
Group 3	39	2	5.12	1 – 17

AF: Absolute frequency; CI: 95% Confidence interval (lower and upper limits).

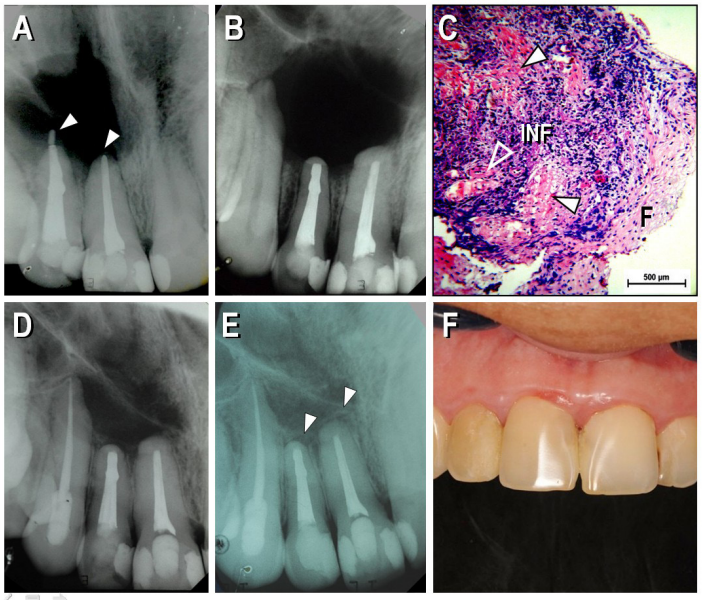

Figure 2: Group 1. A representative case of Group 1 treated with CENP A: Preoperative radiograph of maxillary right central and lateral incisors with large periapical radiolucency showing overfilled root canals (arrowheads). B: Immediate postoperative radiograph. Note the dimensions of the bone cavity after curettage. C: Histopathologic section of the tissue of the lesion showing a periapical granuloma with chronic cell infiltration (INF), wide congested vessels (open arrowhead) and extensive hemorrhagic areas (arrowheads). F: Fibrous capsule. H&E stain. Original magnification x10; Bar: 500 µm. D: 12-month follow-up radiograph. E: After 24 months the lesion had completely resolved (healed). Note that new cortical bone has been formed around the root apex (arrowheads). F: Clinical photograph of the patient at the end of the observation period.

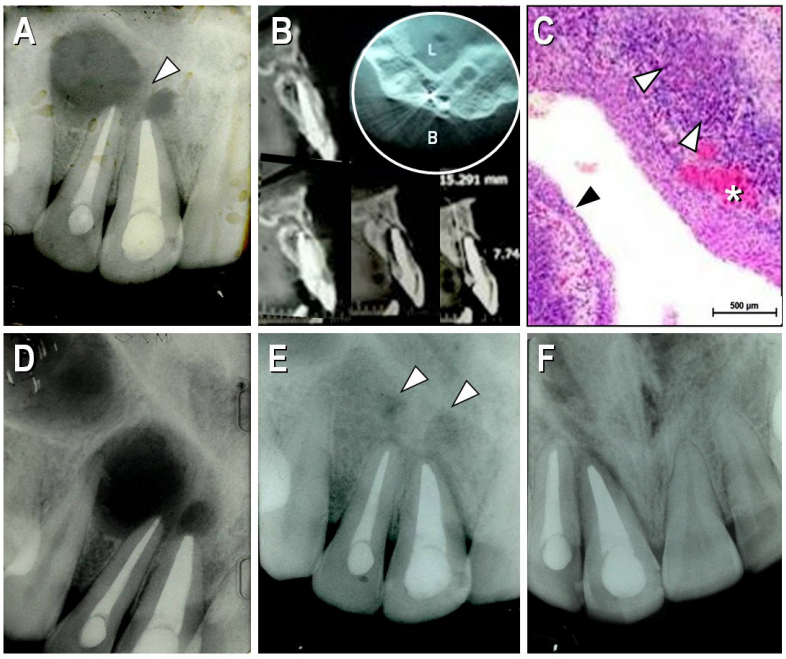

Figure 3: Group 2. A representative case from Group 2 treated with FLM. A: Preoperative PA radiograph of maxillary right central and lateral incisors showing large periapical radiolucency. Note a bridging osseous band (arrowhead). B: Preoperative serial CBCT sections of the periapical lesion taken every 0.5 mm in a sagittal plane showing a thick cortical bone-like tissue surrounding the lesion. Circle: Preoperative CBCT slice of the lesion taken in an axial plane. C: Histopathologic image of the lesion showing a periapical cyst with stratified squamous epithelium (black arrowhead), with surrounding fibro granulomatous tissue (white arrowheads). Note the presence of an hemorrhagic area (asterisk). H&E stain. Original magnification x20; Bar: 500 µm. D: Immediate postoperative PA radiograph after curettage. E: 12-month follow-up PA radiograph. Bone repair is not complete but healing is in progress (arrowheads). F: 24-month follow-up PA radiograph showing complete healing of the lesion.

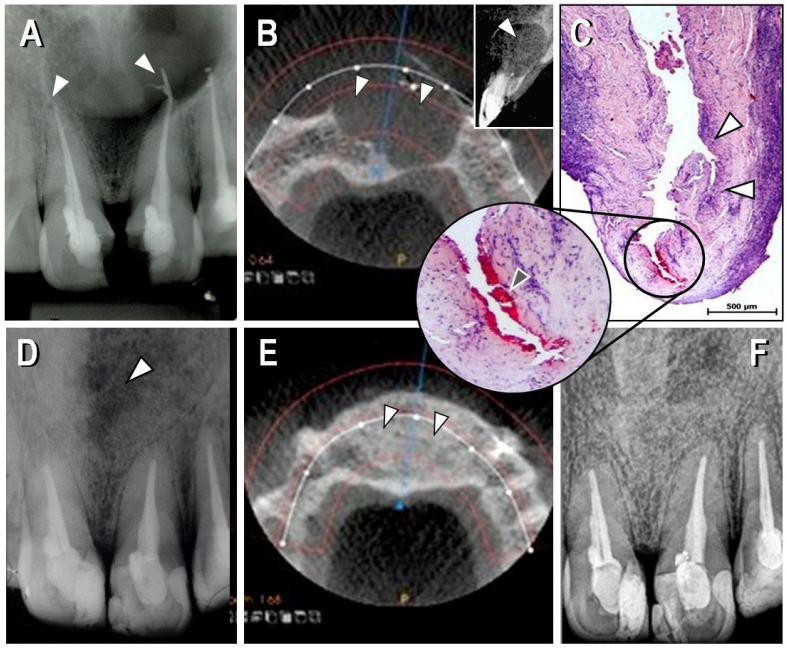

Figure 4: Group 3. A representative case from Group 3 treated with FLM-HBG. A: Preoperative PA radiograph of maxillary right and left central incisors showing considerable periapical radiolucency with overfilled root canals (arrowheads). B: Preoperative CBCT view taken in an axial plane. Note the extensive bone loss caused by the lesion (arrowheads). Insert: Preoperative CBCT view taken in a sagittal plane. C: Histopathologic section of the removed lesion showing a periapical cyst with an inflammatory cell concentration beneath the epithelium (white arrowheads). Insert: Note the presence of an ulcerated area with hemorrhage (black arrowhead). H&E stain. Original magnification x20; Bar: 500 µm. D: 12-month follow-up PA radiograph showing advanced but incomplete bone healing (arrowhead). E: 24-month follow-up CBCT view taken in an axial plane showing complete bone repair of the affected area (arrowheads). F: 24-month follow-up PA radiograph demonstrating complete healing.

Postoperative Signs and Symptoms

Overall after 24 months, postoperative pain was reported in 46.15% (n=18) of failure cases, fistulas in 87% (n=20) and swelling/inflammation in 44% (n=10). In 52% (n=12), a combination of radiolucency, pain, fistula and swelling was observed. The remainder of the failures showed the following combinations: swelling and radiolucency (17%, n=4), pain and radiolucency (9%, n=2) and pain, fistula and radiolucency (35%, n=8). For evaluation of the association with the occurrence of postoperative fistula, patients were pooled into two groups according to the type of treatment: CENP or FLM-HBG. In the group treated with CENP, the presence of a postoperative fistula was observed in 38% (n=15) while in group treated with FLM-HBG a fistula was present in 6% (n=5) of the cases (x2: 19.76 P<0.001, odds ratio: 0.10 with 95% confidence interval 0.03-0.31).

Histopathological Study

Of the total number of biopsies that were submitted for histopathologic evaluation, only the results of the 120 patients that presented for the final 24-month evaluation were included in the study. Microscopic examination of the excised tissues showed the presence of granulomas and inflammatory cysts in subsequently 61% and 39% of the cases. Granulomas consisted of fibro-granulomatous tissues with a chronic inflammatory cell infiltrate, mainly composed of lymphocytes, plasmacytes and macrophages. Enlarged congested blood vessels, hemorrhagic areas and occasional multinucleated giant cells were also observed. In some cases, foci of high concentration of polymorphonuclear neutrophils and abscessed areas were seen. Fragments of spongy bone tissue or calcified chips were occasionally observed in the periphery. On two occasions epithelial cells were seen. The histopathologic observation of periapical inflammatory cysts revealed an internal wall of stratified squamous epithelium surrounded by fibro-granulomatous tissue and the presence of chronic inflammatory cells. A concentration of polymorphonuclear neutrophils was also seen in some ulcerated sectors. Cholesterol crystals were occasionally observed within the cyst cavity.

Discussion

Periapical pathology represents a permanent challenge in dentistry and when not treated in time, it may lead to loss of a tooth. Periapical endodontic surgery is the treatment of choice when primary endodontic treatment and retreatment have failed [22,23]. The recall rate (69%) after 24 months was considered acceptable and met the American Dental Association requirements for subject size in clinical trials [24]. The patients who were not available for the final 24-month evaluation (censored data) had either moved or did not respond to the recall request. Therefore, the results of the biopsies of these patients were not included in the study. It has been reported that the percentage of failures in conventional endodontic surgery of periapical pathologies of different magnitude is approximately 60% [22]. In this study we report on the effectiveness of endodontic microsurgery when resorbable FLM, either individually or combined with an osteoconductive biomaterial such as HBG, was used and compared to CENP. The cases in which the radiolucency had decreased in size but not totally resolved, while being free of clinical signs and symptoms, were considered as healing in progress, because they had the potential to heal over time. Similar to the results reported by Peterson and Gutmann [25], in this study 51% of the cases treated with CENP were successfully resolved after 24 months. However, patients treated with FLM or FLM-HBG showed a higher success rate. Our findings agree with previous studies [26-28], who reported that the use of resorbable membranes in conjunction with bone grafts enhanced bone regeneration. Successful endodontic microsurgery includes regeneration of bone, periodontal ligament, and cementum. In many cases, incomplete healing due to ingrowth of connective tissue prevented the regeneration of periapical tissues. Recent technological advances in surgical techniques have significantly decreased the failure rates. With the objective to improve success, the use of biocompatible barriers has been promoted [3,29,30]. In endodontic surgery, different biomaterials have been used as osteoconductive scaffolds [30-32], but none have obtained universal acceptance among researchers. GBR with the use of biomaterials such as bioresorbable membranes and bone substitutes is presently successfully being used in surgical protocols [32]. Perelman et al. [33] demonstrated that the combined application of mineralized bovine bone and collagen membranes increased bone regeneration compared to that in patients treated with the bone substitute alone. In the present study, the use of membranes and lyophilized human bone grafts in patients of Group 3 achieved a success rate of 95%. Although a higher percentage of success was recorded in Group 3, the differences with Group 2 were statistically insignificant. Thus, in agreement with previous reports [26,28-31], our results showed that the use of either FLM or FLM-HBG in endodontic surgery promoted bone repair. The FLM acts as a biological barrier between the bone and the mucosal tissue during the critical time of bone repair, while the HBG forms a “scaffold” which accelerates the regeneration process. As a biological barrier, the FLM acts between 4 and 6 weeks, is biocompatible and allows for a fast vascularization. In addition, the FLM is malleable and adapts easily to bone defects [4,9]. It should be emphasized that for this study smokers and diabetic patients fall within the exclusion criteria. Smokers are at significantly higher risk of post-surgical complications including impaired heart and lung functions, infections and delayed or impaired wound healing [34]. In diabetic individuals complexity arises due to an elevated risk of complications on the treatment outcome including delayed wound healing necessitating a constant blood sugar control, judicious antibiotic use, special postoperative care and interaction with external healthcare providers [35].

In previous studies [15,21], CBCT scans showed a more relevant anatomic information than PA radiographs for preoperative planning as well as for postoperative evaluation of periapical surgery. However, the results of the current study surprisingly agreed with those of Gurutami et al. [36] who found that PA and CBCT scans did not show significant differences for assessing the healing outcomes of endodontic microsurgery. In agreement with García et al. [37], the histopathologic study of the removed lesions revealed that the most prevalent periapical lesions were

subsequently apical granulomas followed by inflammatory cysts and granulomas containing epithelial cells. As per protocol, the inclusion criteria required that the selected cases must show periapical lesions involving at least two apexes as shown on preoperative PA radiographs. Relevant to the prevalence of periapical lesions associated with endodontic failures, our findings agree with Çalişcan et al. [38] and Kharat et al. [39] but disagree with the results of Banomyong et al. [40], who suggested that the prevalence of periapical cysts over granulomas increased as the size of the radiolucency increased and/or involve the adjacent teeth. These differences may be due because in this study we used a different protocol design and a high number of treated patients.

Conclusion

Within the limits of the present study, the results tend to support the use of FLM and FLM-HBG as the recommended treatment modality for endodontic microsurgery of periapical pathologies.

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