10.21608/adjc.2025.360531.1701 Advanced Dental Journal
Volume 7 (2025) | Issue 3| Pages 448-456 ©Faculty of Dentistry-Cairo University

Original Article

Silk Fibroin Nanofiber Membrane Using Minimally Invasive Surgical Technique in Management of Grade II Furcation Involvement: A Randomized Clinical Trial "Silk Fibroin in furcation"

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Submitted: 23-02-2025
Accepted: 14-05-2025

Abstract

Aim: To investigate the clinical and radiographic outcome of the regenerative treatment of molars with grade II furcation involvement (FI) using minimally invasive surgical technique (MIST) with silk fibroin (SF) membrane in comparison to MIST. The research is planned as a prospective, controlled, single-blind, randomized clinical experiment.

Patients and methods: A total of twenty patients with Grade II FI were randomly assigned to two groups; Group I: MIST (n=10); Group II: MIST+ SF membrane (n=10). Clinical (Clinical Attachment Level [CAL], Probing Pocket Depth [PD], Plaque Index [PI] and Gingival index [GI]) and radiographic (filled bone volume [FBV]) parameters were evaluated at baseline and 6 months postsurgical. FBV was the primary outcome.

Results: For both groups, a statistically significant improvement was reported for CAL (1.63 ± 1.02, 2.75 ± 0.65), PD (1.63 ± 0.68, 2.25 ± 0.47) and FBV (0.02 ± 0.07, 0.056 ± 0.062) for control and test group respectively, at 6 months evaluation period. There was an insignificant difference between the study groups.

Conclusions: The addition of SF membrane to MIST improved clinical and radiographic outcomes when treating molars with degree II FI, however, this improvement was statistically insignificant.

Keywords: Grade II furcation involvement, Periodontal regeneration, Silk fibroin, Minimally Invasive Techniques, Cone Beam Computed Tomography

Introduction

The chronic, multifactorial inflammatory illness known as periodontitis is brought on by the host's inflammatory response to a subgingival microbiota, which results in the loss of the alveolar bone and connective tissue connection, which support teeth. Prolonged inflammation can lead to alveolar bone loss, which can spread to the furcation regions if treatment is unsuccessful ***(Abou El Fadl et al., 2021)***.

The degree of periodontal tissue loss determines the classification of furcation problems. Class I refers to a loss of horizontal periodontal attachment support that is less than one-third of the furcation area's overall width, while class II refers to a loss of horizontal attachment that is more than one-third. Class III refers to lack of horizontal attachment ***(Hamp et al., 1975)***. Regenerating the furcation defect presents two difficulties. First, it requires the involvement of several cell populations in the regeneration of both soft and hard tissues ***(Nibali et al., 2018)***. Second, full periodontal wound healing or regeneration is not anticipated after conventional therapies due to the uneven and complicated architecture of furcation ***(Rasperini et al., 2020).***

The prognosis of teeth affected by furcation has been improved using a variety of techniques, including guided tissue regeneration (GTR), resective osseous surgery ***(DeSanctis and Murphy, 2000),*** open flap debridement, and scaling and root planing. The periodontal defect is fully covered and encircled by a barrier membrane in GTR. This prevents the epithelium and gingival connective tissue from migrating apically to the defect and maintains the area beneath the membrane, which promotes the regeneration of bone and new periodontal ligaments ***(Jepsen et al., 2002).***

Due in large part to the periodontium's inherent capacity for healing, full regeneration of teeth with furcation involvement (FI) utilizing GTR is frequently unexpected ***(Sanz and Giovannoli, 2000).*** Furthermore, FI regions that are bigger than 4 mm in height often have a much lower success rate when using the GTR technique ***(Nibali et al., 2018)***. Several scaffolds were employed to improve the characteristics of GTR membranes in order to raise the success rates of GTR ***(Geão et al., 2019).***

Scaffolds must possess the qualities required for safe tissue usage as well as those that improve the healing potential in order for tissue regeneration to be effective. The first need is for biocompatibility. However, for cells and nutrients to enter, the scaffolds must have the right mechanical characteristics, an acceptable rate of degradation, and sufficient permeability and porosity ***(Geão et al., 2019).***

Silk fibroin (SF) is a natural protein extracted from the cocoons of the silkworm. It can be fabricated within a nanoscale. SF nanofiber is suggested to be used in GTR because of its favorable properties including biodegradability, bioavailability, good cell adhesion, intrinsic antimicrobial properties and adequate mechanical properties ***(Kwang-Jun and Hyun, 2018).*** Excellent osteogenic cell differentiation, proliferation, and new bone regeneration are displayed by the SF membrane ***(Wei et al., 2011).***

The capacity of the surgical technique to establish and preserve sufficient space for the blood clot and to give it stability and protection through primary closure are crucial components of effective periodontal regeneration ***(Nishat et al., 2020).*** Less invasive clinical techniques and flap designs have been developed recently. These techniques, which limit periodontal tissue damage, improve clot stabilization, improve soft tissue stability, and lower patient morbidity, are based on the use of papilla preservation flaps and microsurgical tools. When compared to traditional surgical methods, the use of such minimally invasive treatments has shown better results ***(Cortellini et al., 2019).***

In this study, were investigated clinical and radiographic outcome of the regenerative treatment of molars with grade II FI using MIST with SF membrane in comparison to MIST. To the best of our knowledge, this is the first study that introduces SF membrane as GTR membrane for treating furcation defects.

Subjects and Methods

Sample size calculation: The clinical outcome of MIST [group 2 (G2)] with SF membrane was compared to MIST alone [group 1 (G1)] for the management of grade II furcation defects, FBV of the furcation, as measured by CBCT 6 months after surgery, was selected as a primary outcome and a total sample size of 20 (10 in each group) was sufficient to detect difference of 0.5 mm between treatments with a significance level of 5% and a power of 80%. A 20% dropout was estimated ***(Mallapragda et al., 2024).***

Study design, subject population, and compliance with ethical standards: The research was planned as a prospective, controlled, single-blind, randomized clinical experiment.

The study evaluated the SF nanofiber membrane's regeneration potentials for the regenerative therapy of grade II FI both clinically and radiographically using minimally invasive surgery. Twenty patients with periodontitis were gathered from the outpatient clinic of the Faculty of Dentistry's Oral Medicine and Periodontology department at October 6 University. The proposal was reviewed and approved by the Faculty of

Dentistry, October 6 University Research Ethics Committee (RECO6U/21-2023). Signed informed consents were obtained from all patients after being informed about study protocol and the surgical procedure including objectives, study visits, risks, and benefits. The data obtained from patients as well as the results of the follow up were kept confidential.

Inclusion and Exclusion criteria: Patients were selected based on the following criteria: 1) Age between 30 and 65 years old; 2) Systemically healthy based on medical health questionnaire modified from Burkitt's in oral medicine**;** 3) history of compliance with oral hygiene instructions and periodontal recall; 4) Periodontitis stage III or IV ***(Tonetti et al., 2018);*** 5) Grade II Glickman FI; A cul-de-sac as there is a definite horizontal component defect (HCAL ≥3 mm) and with PD ≥5 mm in the mid-buccal or mid-lingual sites when evaluated 6 weeks after non-surgical periodontal therapy. The interdental bone had to be coronal to the furcation entrance; 6) No mobility. The following patients were excluded: 1) patients with systemic illness known to affect the outcome of periodontal therapy, including diabetes, immune deficiency, osteoporosis, anemia and viral infection; 2) patients on drugs including corticosteroids, bisphosphonate, anticoagulants and antiplatelets within the past three months; 3) patients under treatment of chemotherapy or radiotherapy. 4) pregnant and lactating women; 5) history of periodontal surgery at the involved sites.

Fabrication and characterization of Silk Fibroin (SF) nanofiber membrane

SF nanofiber membrane: the protein was extracted from its natural source; silkworm (Bombyx mori). Sericin in the cocoons was de-gummed by being boiled with calcium carbonated and the fibroin was treated using lithium bromide to be finally dialyzed against water. The molecular weight was determined using sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. For the synthesis of the nanofiber membrane: electrospinning was the adopted method for the nanofiber fabrication ***(Kim et al., 2003).*** The resulting membrane exhibited a uniform fiber morphology with an average pore size of approximately 100 μm, thickness of about 300 μm and fiber diameter of 150 nm to 250 nm, providing an optimal scaffold for guided tissue regeneration. Surface analysis revealed a porous structure with high surface area and favorable hydrophilic properties, which enhance cell attachment and nutrient diffusion. The membrane density and mechanical strength were within the suitable range for periodontal applications ***(Ko et al., 2016).***

Preoperative preparation: All subjects were screened by comprehensive periodontal examination and full periodontal charts were obtained. Initial therapy included a full mouth supra- and sub-gingival debridement, oral hygiene instructions, and dental prophylaxis. One-month evaluation of adequate oral hygiene and control of gingival inflammation was performed before surgical decision. All clinical parameters were reexamined to ascertain the suitability for the surgery.

Clinical assessment: Six weeks' evaluation of adequate oral hygiene and control of gingival inflammation (PI <15%) and (BI <10%) was achieved before surgical procedures. Clinical parameters including CAL, PPD, PI and GI were documented on the day of the surgical appointment immediately prior to surgery (baseline) and 6 months post-operatively.

Radiographic assessment: CBCT was performed using Planmeca ProMax® 3D Mid Machine with Planmeca Romexis Viewer 4.4 software, to evaluate the vertical bone depth (VD) and FBV at the furcation area. VD measured from a line runs vertically from the furcation fornix to the middle of the defect base. While FBV measurements were taken by segmentation as multiple segments were taken in the defect distance from the buccal surfaces of the roots to the lingual surfaces. CBCT for the sites was performed at baseline (the day before the surgical procedure), and 6 months post-operatively.

Surgical phase: Prior to the surgical procedure, the patients were instructed to rinse with 0.12% chlorhexidine gluconate mouth wash (Hexitol 0.12%; Arabian delta company (Adco). At the selected surgical site, adequate anesthesia (Mepicaine-L) was given using nerve block technique.

Surgical procedure of group 1: A Minimally invasive papillary preservation flap (MIPPF) and sulcular incisions were done with a 15C Bard –Parker surgical blade and was elevated by blunt dissection using a periosteal elevator. After removing the granulation tissue from the furcation defect and thoroughly debriding the area using site-specific Gracey curettes to guarantee a clean site, the surgical site was washed for 20 seconds with sterile saline solution. The flap was secured with vertical mattress sutures placed in the interproximal spaces mesially and distally using size 5-0 non-resorbable polypropylene suture**.**

Surgical procedure of group 2: The same surgical procedure was performed for group II (test group), except for SF membrane placement over the defect**.** The membrane trimmed and adapted over the furcation defect and extends from the CEJ to 3 mm beyond the furcation defect, so as to provide a broad base during the placement.

Post-operative regimen: Written and spoken postoperative instructions were given to every patient. Patients were instructed to put a cold pack extra oral to the surgical site immediately after surgery and for the whole first day for 10 mins in a different period. They were instructed to avoid tooth brushing and interdental cleaning at the surgical site, and to clean the surgical sites with a cotton pellet soaked in saline for 3 weeks, and to rinse twice daily with a 0.12% chlorhexidine digluconate mouth rinse for 14 days. For postoperative medications patients were prescribed amoxicillin (500 mg) every 8 hours for 1 week. Patients allergic to prescribed medication and derivatives were prescribed clindamycin 300 mg every 8 hours. For postoperative analgesia, 600mg Ibuprofen 2 times/d for 2 days was prescribed**.** In order to remove the sutures, patients were visited at the end of the first week and again at the end of the second week. After one, three, and six months, patients were brought back for prophylaxis. Guidelines for maintaining good oral hygiene were reinforced, and if necessary, supragingival scaling was carried out at each visit. Six months after surgery, all clinical and radiological characteristics were re-recorded for each patient in the research.

Statistical analysis: Statistical analyses are performed using Statistical Package for Social Sciences (IBM SPSS Statistics version 26). Numerical variables express by mean, standard deviation and range, nominal variables are expressed using median, frequency and percentage. The tests are used in this analysis: The normality test is used to examine the normality of the data and for parametric variables: The independent t-test is used to compare difference between the studied groups. The Paired t-test is used to compare the difference between before and after treatment at each group. For non-parametric variables: Mann-Whitney test used to compare difference between the studied groups. Wilcoxon test used to compare the difference between before and after treatment at each group.

Results

Clinical assessment: A total of 20 patients, 9 females and 11 males ranging in age from 36 to 52 (mean 40 years), with 20 molars with grade II FI were evaluated in this study. The 20 furcation sites included mandibular molar teeth (12 sites / 60%) and maxillary molar teeth (8 sites / 40%).

All the patients complied with the established protocol for the entire length of the study, there were no adverse reactions or serious complications reported for the SF membrane or the surgical procedures. Almost all patients were satisfied with the surgical outcome.

Changes in clinical parameters are reported in Table 1. PI and GI values shows insignificant intra- or intergroup difference ($P>0.05$) after 6 months. At baseline, mean ± SD of CAL in G1 and G2 was 5.88±1.13 and 6.13 ± 0.84 respectively. After 6 months, CAL was reduced to 4.25±1.03 and 3.38 ± 0.52 for G1 and G2 respectively. There was insignificant difference between both groups at both evaluation periods. In each group, a statistically significant PD reduction from 5.38 ± 0.74 and 5.50 ± 0.54 at baseline to 3.75 ± 0.71 and 3.25 ± 0.46 at 6 months was recorded for G1 and G2 respectively. However, the comparison of PD reduction between groups yielded insignificant difference at baseline ($P = 0.705$) and after 6 months ($P = 0.116$).

Radiographic evaluation by CBCT: Changes in radiographic parameters are reported in **Table 1**. At linear VD measurement (FF – middle of defect base) there was a significant decrease in G2 from 1.85±0.45 at baseline to 1.55±0.47 after 6 months, while there was insignificant difference between groups. Regarding FBV, there was a significant difference between baseline and 6 months values in G1 ($p = 0.023$*) and G2 ($p = 0.001$*), while no significant difference was reported between the studied groups after 6 months ($p = 0.605$).

Table (1): Clinical and radiographic parameters for both groups at baseline and follow-up

Variable		Control group (MIST)		Test group (MIST with SF Membrane)		p-value
		Median	Mean	Median	Mean	
GI	baseline	0.25	0	0	0	0.143
	After 6 months	0.13	0	0	0	0.317
	P-value	0.564		0.564		-------
PI	baseline	0.25	0	0	0	0.143
	After 6 months	0.38	0	0	0.25	0.602
	P-value	1.000		0.157		-------
		Mean ± S.D	Range	Mean ± S.D	Range	
PPD	baseline	5.38±0.74	5—7	5.50 ± 0.54	5—6	0.705
	After 6 months	3.75±0.71	3—5	3.25 ± 0.46	3—4	0.116
	P-value	0.000**		0.000**		------
CAL	baseline	5.88±1.13	5—8	6.13 ± 0.84	5—7	0.622
	After 6 months	4.25±1.03	3—6	3.38 ± 0.52	3—4	0.057
	P-value	0.000**		0.000**		-------
VD	baseline	1.84±0.52	1.20—2.75	1.85±0.45	1.27—2.34	0.964
	After 6 months	1.64±0.34	1.17—2.19	1.55±0.47	1.08—2.18	0.657
	P-value	0.141		0.002*		--------
FBV	baseline	0.104±0.08	0.03—0.24	0.155±0.08	0.03—0.24	0.233
	After 6 months	0.084±0.07	0.03—0.20	0.099±0.05	0.02—0.16	0.605
	P-value	0.023*		0.001*		--------

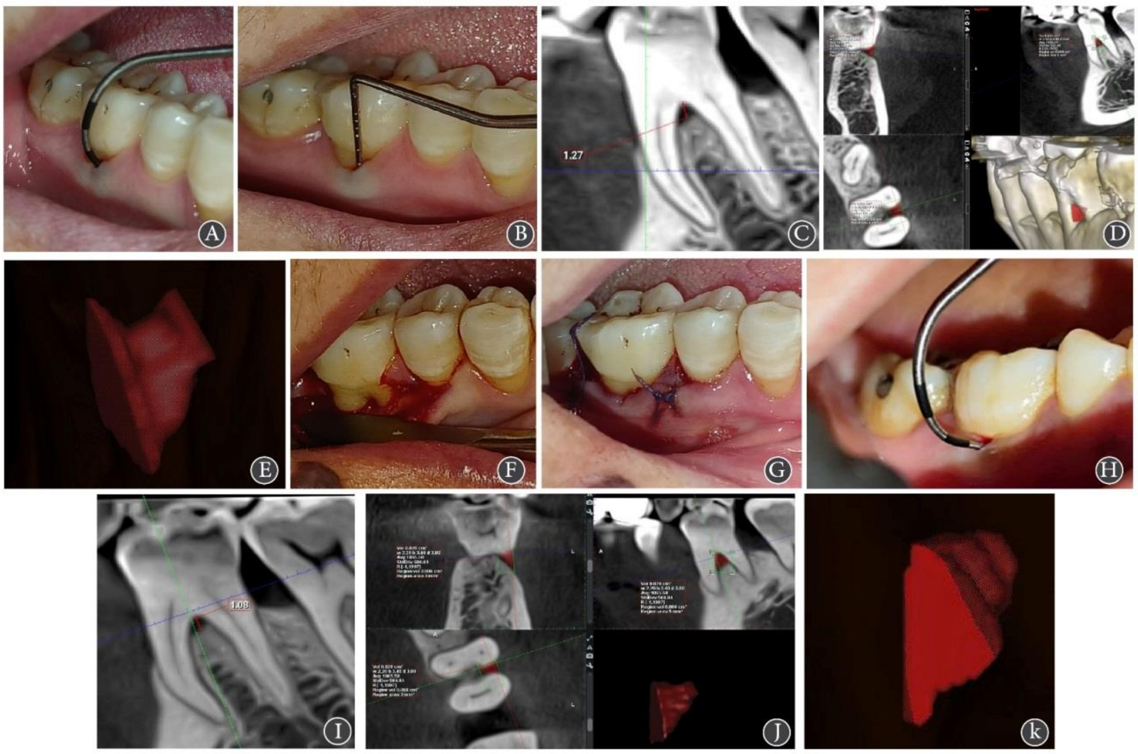

Figure 1: A) Grade II FI assessed using Naber's probe, B) PPD Measurement for buccal aspect of the lower right first molar, C) CBCT sagittal cut showing VD linear measurement of the furcation defect at buccal aspect before surgery, D) CBCT multiple cuts showing the furcation defect volume from different aspects, E) 3D shape showing the furcation defect volume, F) MIPPF and granulation tissue debridement, G) Flaps repositioned and sutured, H) 6 months follow up after surgery, I) CBCT sagittal cut showing VD linear measurement of the furcation defect at buccal aspect 6 months after surgery, J) CBCT multiple cuts showing the furcation defect volume from different aspects 6 months after surgery, K) 3D shape showing reduction of the furcation defect volume 6 months after surgery.

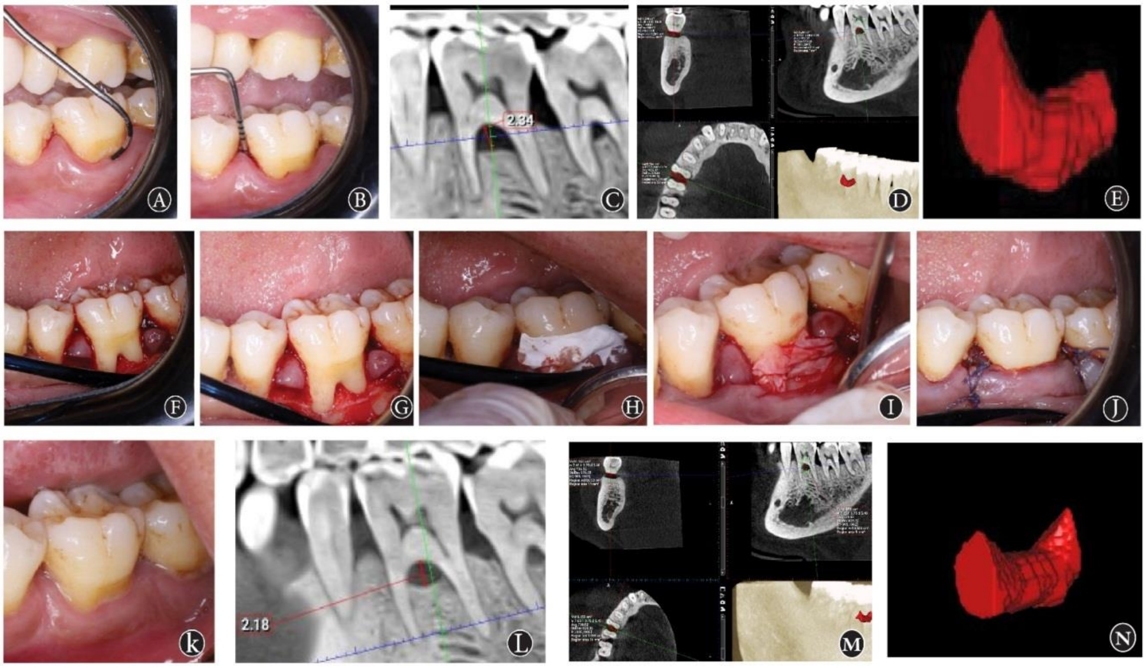

Figure 2: A) Grade II FI assessed using Naber's probe, B) PPD Measurement for mesial aspect of the lower right first molar, C) CBCT sagittal cut showing VD linear measurement of the furcation defect at buccal aspect before surgery, D) CBCT multiple cuts showing the furcation defect volume from different aspects, E) 3D shape showing the furcation defect volume, F) MIPPF, G) Granulation tissue debridement, H) Application of SF membrane at the furcation defect, I) SF membrane adapted into the defect, J) Flaps repositioned and sutured, K) 6 months follow up after surgery, L) CBCT sagittal cut showing VD linear measurement of the furcation defect at buccal aspect 6 months after surgery, M) CBCT multiple cuts showing the furcation defect volume from different aspects 6 months after surgery, N) 3D shape showing reduction of the furcation defect volume 6 months after surgery.

Figure 3: Prepared SF nanofiber membrane ready to use

Discussion

This study was designed to determine whether GTR with SF membrane may enhance the outcomes of MIST in the management of class II FI. According to the current study's results, the test group and control group did not differ significantly in terms of GI, PI, CAL, and PD at baseline or at 6 months (P<0.001), but both groups experienced a significant decrease in PPD and an increase in CAL from baseline to 6 months, as well as a significant FBV for both groups' sites. The two groups did not, however, vary significantly.

Although upper and lower molars exhibit anatomical and regenerative differences, with maxillary molars generally presenting more complex furcation morphology and less favorable outcomes, both were included to broaden the clinical applicability of the findings and to evaluate the performance of the SF membrane across a range of clinical challenges. The inclusion of both arches also reflects real-world scenarios and helps assess the consistency of treatment outcomes in diverse anatomical contexts ***(Dorje et al. 2021).***

Good oral hygiene is a must before surgical procedures and throughout the study period. We evaluated PI and GI. This allowed us to include oral hygiene as a factor in cases of inferior results. Both treatment modalities demonstrated optimal PI and GI at base line and 6 months evaluation period.

In terms of PD, both groups saw a decrease from baseline to 6 months, CAL gain was achieved, which may be explained by full debridement as a result of genuine periodontal regeneration by new attachment or healing by repair. The addition of SF membrane to MIST improved clinical and radiographic outcomes when treating molars with degree II FI, however, this improvement was statistically insignificant when compared to MIST alone (p=0.116). These results may be attributed to the characteristics of the included furcation defects (shallow grade II FI) making the benefits of adding the membrane insignificant when compared to the MIST alone. Furthermore, it is impossible to rule out the possibility that adding bone replacement grafts (BRG) might enhance the outcomes and stop membrane collapse which cannot be prevented in this investigation ***(Jepsen et al., 2020).***

The current results agreed with the study of ***Wang et al. (Wang et al., 1994)*** who compared absorbable collagen membrane in treating grade II FI versus OFD and concluded that both control and test groups demonstrated significant differences in PD and CAL while no significant difference between them. Furthermore, when furcation involvement and profound intrabony abnormalities coexist, the work by ***Cortellini et al. (Cortellini et al., 2019)*** shown that MIST, even in the absence of regenerative materials, can offer therapeutic improvements to molars that are significantly impaired. The authors did, however, establish that the results were achieved in people who were well-maintained and cooperative, and who had an interdental peak of bone and gingival edge coronal to the furcation entry.

On the contrary, this study does not coincide with ***Khanna et al.,*** who found that there was a significant difference between test group and control group in management of grade II FI and that may be due to the use of bone graft with collagen membrane versus OFD in control group ***(Khanna et al., 2012).***

Due to the wide anatomical variance in furcation topography including trunk length, depth and location of concavities, and root divergence, the furcation defect is difficult to quantify, with a multitude of confounding factors related to molar furcation anatomy and inability to instrument properly. CBCT is a way to evaluate this three-dimensional defect space because it may confirm hard tissue fill and success of treatment. The SF membrane's porous nature, which promoted the attachment, proliferation, and differentiation of human mesenchymal stem cells, may have contributed to the test group's significant decrease in VD between baseline and six months postoperative, according to the current study's CBCT results ***(Mobini et al., 2013)***. Between baseline and six months, there was a significant decrease in FBV in both research groups.

Clinical management and prognosis of the furcated molar is truly dependent on how advanced the defect has invaded horizontally. Therefore, measuring horizontal probing depth is highly recommended in the upcoming studies. Additionally, in future studies, the relation between the level of the interdental bone and gingival margin, and the furcation defect should be evaluated in relation to the obtained results. Better results are usually expected when the gingival soft tissue margin and interdental bone are coronal to the furcation entrance.

Moreover, clinical attachment level gains and probing depth may not be the best parameters to use when comparing different regenerative therapies against each other. Since this is a closed soft tissue measurement, the probe will stop where the tissue attachment allows. If healing is mostly done by repair and a long junctional epithelium and not with true regeneration in the defect, the clinical probing is not an accurate form of measure for clinical success. Therefore, CBCT to confirm hard tissue fill is highly recommended.

Conclusion

To the best of our knowledge, this study was the first clinical study that used SF nanofiber membrane to treat furcation defects. Future evidence may validate its safety and use in routine regenerative therapy.

Acknowledgment

The authors would like to acknowledge and thank **Assoc. Prof. Sara A. Abdel Gaber** (Nanomeidicine Department, Institute of Nanoscience and Nanotechnology, Kafrelsheikh University, Egypt) and **Dr. Salma nasser Hassan** (Institute of nanoscience and nanotechnology at kafrelshiekh university) for formulation of the silk Fibroin membrane that was used in this study.

Conflict of Interest

The authors had no conflict of interest concerning the topic under consideration in this article.

Funding

The authors disclose no financial relationships relevant to this article.

Ethical Approval

All procedures performed were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments. The clinical trial was approved from October 6 University, Faculty of Dentistry research ethics committee (RECO6U/21-2023).

Availability of Data

All supporting data are available on request.

Author Contributions

All authors had substantial contributions for conception, design, acquisition of data, analysis, interpretation of data, the drafting of the manuscript and critical revision and final approval for its intellectual content.

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