

Efficacy of recombinant human fibroblast growth factor 2 impregnated absorbable collagen membrane in the treatment of Miller's Class I and II gingival recession defects Preliminary results from the first in human clinical trial

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Abstract:

Aims: This study was a single-arm trial to obtain preliminary data on the efficacy of collagen membranes impregnated with recombinant human fibroblast growth factor-2 (rhFGF-2) in the treatment of Miller's Class I and II gingival recessions. **Materials and Methods:** Twenty-one individuals (34 sites) presenting with localized Miller's Class I and II gingival recessions were included in this study. Following a standard surgical protocol, rhFGF-2-impregnated membranes were placed in sites with gingival recession. Clinical parameters such as width of keratinized gingiva (wKG), recession depth (RD), and probing depth were measured at baseline and after therapy completion at 3 and 6 months. **Results:** Most of the sites exhibited favorable clinical healing; the most common complications were persistent edematous and inflamed gingivae beyond 1 week ($n = 3$), development of residual periodontal pockets ($n = 2$), and no reduction in RDs ($n = 2$). Significant improvements in wKG and RD were noted from baseline to 6 months. **Conclusion:** rhFGF-2-impregnated collagen membranes showed promising results in terms of increasing the wKG and recession coverage. A comparison with other standard therapies and agents in subsequent trials may shed more light on the clinical efficacy of this material.

Key words:

Fibroblast growth factor-2, gingival recession, periodontal atrophy

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INTRODUCTION

Gingival recession is the apical displacement of gingival margin resulting in exposure of root surface;^[1] the etiology of the condition is multifactorial and may include periodontal disease, microbial deposits, inflammation, improper flossing, aggressive toothbrushing, incorrect occlusal relationships, and anatomical deformities.^[2] It was suggested by several authors that gingival recession occurs in patients with a thin gingival biotype.^[2-4] Seibert and Lindhe^[2] classified the gingiva into "thick-flat" and "thin-scalloped" biotypes. A gingival thickness of greater than 2 mm is considered as a thick tissue biotype and *vice versa*.^[4,5]

Soft tissues can be regenerated to cover root exposure, and a thin biotype can be converted into a thick biotype using recombinant human growth factor technology.^[6-8] A recent review stated

that growth factors could enhance soft-tissue regeneration which includes restoration of mucogingival architecture and regeneration of periodontal hard and soft tissues including bone, cementum, and periodontal ligament fibers.^[1,8,9]

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Fibroblast growth factor-2 (FGF-2), a heparin-binding cytokine with strong angiogenic activity, stimulates the proliferation of undifferentiated mesenchymal cells.^[10] These functions can be applied in mucogingival surgery as FGF-2 promotes bone^[10] and cementum^[11] formation and exhibits an increased potential to promote periodontal regeneration in recession defects.^[11,12]

However, to the authors' knowledge, the treatment of gingival recession using recombinant human FGF-2 (rhFGF-2) in humans has not been reported. The aim of this study was to evaluate and validate the clinical efficacy of rhFGF-2-impregnated absorbable collagen membrane in the treatment of Miller's Class I and II gingival recession defects.

MATERIALS AND METHODS

Sample size

From pilot trials, changes in width of keratinized gingiva (wKG) by the membrane as compared to a surgical control at 1 month were utilized to calculate the sample size. A mean difference (μ) of 1.02 mm was seen at 1 month between both the groups with a standard deviation (SD) (σ) of 0.46 mm. Mean differences by their SD. The effect size through μ/σ was 2.2; the minimum sample size for the given clinically significant effect size was calculated through the formula $N = AB/(E/S)^2 = 21$ where $A = (1/q_1 + 1/q_0) = 67.682$ (q_1 and q_2 are the proportion of participants in the membrane/control groups in pilot trial); $B = (Z\alpha + Z\beta)^2 = 7.849$ where $Z\alpha = 1.960$ and $Z\beta = 0.842$ are normal deviations for α and β ; $E = 2.3$; and $S = SD = 0.46$ mm. Thus, a minimum sample size of 21 sites was required to discern an effect of 1 mm at all time frames.

Study methodology

Figure 1 depicts the follow-up scenario and the number of participants at each stage of the study. The trial was planned as a single-arm trial to obtain preliminary data on the efficacy of collagen membranes impregnated with rhFGF-2. Thirty-six systemically healthy controls between 20 and 55 years presenting with Miller's Class I or II gingival recession^[13] were initially screened by the study team [Figure 2a]. Exclusion criteria comprised (1) recessions associated with root demineralization/caries, deep cervical abrasion, or pulpal pathology; (2) patients with a history of systemic conditions affecting the periodontium; and (3) smokers. From this initial patient pool, 34 sites in 21 individuals satisfying the inclusion criteria were selected from the outpatient section of the department of periodontology. All participants provided informed consent, and the study protocol was approved by the institutional ethics committee.

Preparation of the material

Collagen membranes incorporating 10 ng/ml human recombinant basic FGF (FGF-2/bFGF) were prepared as follows. Briefly, standard collagen suspension was produced from type 1 collagen from bovine Achilles tendon by homogenizing the material in 10 mM Na-butyrate solution (Pro Lab Marketing Pvt. Ltd., New Delhi, India). bFGF was reconstituted in 0.1M phosphate buffer and was added to the suspension. Cross-linking of collagen was promoted by adding 0.16% of glutaraldehyde aqueous solution (Sigma Aldrich Chemicals Pvt. Ltd., Bangalore, India), and the resultant solution was placed in individual 1.5 cm × 1.5 cm and 3 cm × 2 cm vats which were maintained at 4°C for 12 h for gelatin cross-linking. The impregnated and cross-linked membranes were dried and placed in ethylene oxide sterilizer (EtO sterilizer, Krishna Engineering, Ahmedabad, India)

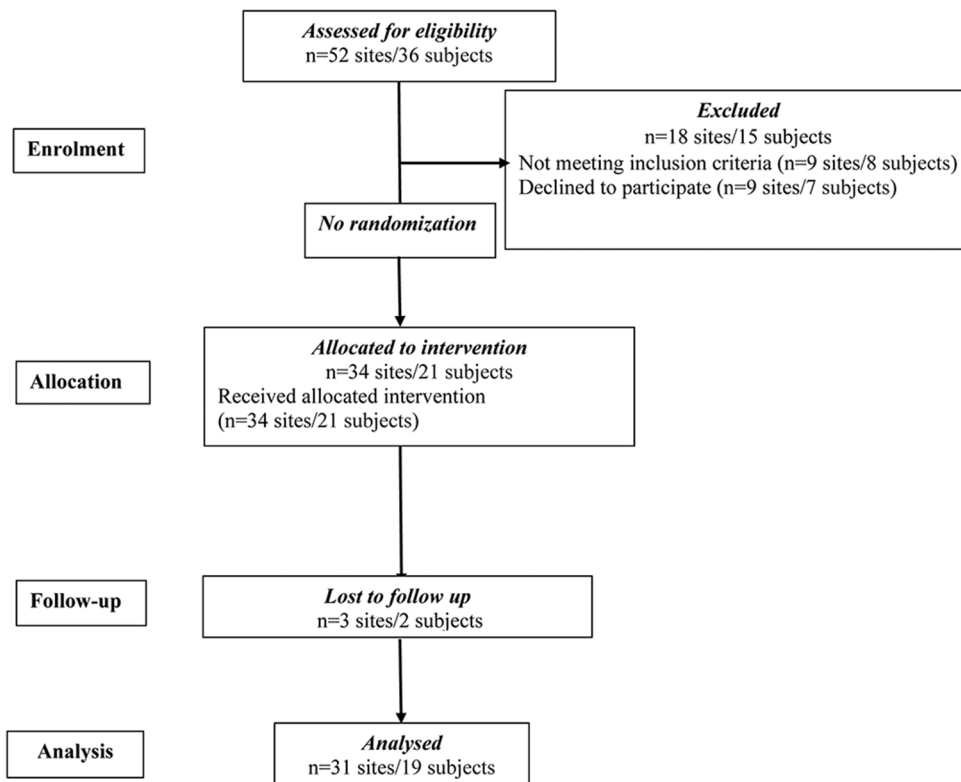


Figure 1: Flowchart depicting the follow-up scenario and the number of participants at each stage of the study.. n – number

for EtO degassing. Aseptic packaging was done as follows; the sterilized scaffolds were freeze-dried in a commercially available laboratory freeze dryer (Lyophilization Systems Pvt. Ltd., Hyderabad, India). After 3 days of Lyophilization, the materials were packaged into sizes 1.5 cm × 1.5 cm and 3 cm × 2 cm.

Procedure

A single designated operator (reverse voice channel) performed all surgical procedures for the sake of uniformity. Two weeks after the completion of initial therapy, the surgical procedure was planned under local anesthesia. A full-thickness mucoperiosteal flap preserving the buccal interdental papillae were raised [Figure 2b]. De-epithelization of the papillae was done. The rhFGF-2-impregnated membrane was placed upon the recession site [Figure 2c] and was stabilized by suturing it to the lingual papillae using 4-0 absorbable sutures [Figure 2d]. The flap was coronally advanced as far as possible to cover the membrane [Figure 2e] and was sutured to the buccal interdental papillae with 4-0 absorbable sutures (Trulene™, Healthium Medtech Pvt. Ltd., Bangalore, India). Postsurgical instructions were explained, and oral antimicrobials (Novamox-500®; amoxicillin 500 mg, TID) and analgesics (Diclomol®; diclofenac sodium 50 mg + paracetamol 325 mg, BID) were prescribed for 5 days postoperatively. Patients were instructed to rinse gently the surgical sites with 10 ml of essential oil mouthwash (Listerine®, Johnson & Johnson, Mumbai, India) twice daily postsurgically after 1 week. They were advised to avoid chewing, brushing, and mastication at the site for 10 days; suture remnants were

removed after 1 week. Oral hygiene instructions were reinforced. Patients were then monitored until the end of the study period.

Measurement of the parameters

The following parameters were measured at each site:^[12,13] (1) wKG – to assess the wKG, the mucogingival junction was identified visually as the border between the movable (alveolar mucosa) and immovable tissues (gingiva). The distance from the gingival margin to the mucogingival junction was considered as the wKG; (2) the distance from the cement-enamel junction to most apical extension of gingival margin was the recession depth (RD); and (3) probing depth (PD) was the distance from the gingival margin to the bottom of the gingival sulcus. All parameters were measured at baseline (before surgery) and at 3 and 6 months after the procedure using a graduated periodontal probe.^[12] The baseline and postoperative outcomes were recorded by three calibrated investigators (YSHSC, KS, and SP); their mean weighted inter-examiner kappa scores were 0.70 ($F = 2.02$; $P = 0.04$), 0.79 ($F = 6.89$; $P = 0.006$), and 0.80 ($F = 1.69$; $P = 0.05$) for wKG, RD, and PD from 10 standardized sites, respectively. As this is a single-arm study, masking was not possible as all investigators were periodontists and sites would be distinct enough to identify interventions.

Statistical analysis

Data were analyzed by Prism 6.0® (GraphPad, La Jolla, USA) and SAS 9.3® (SAS, Mumbai, India). Data were summarized by mean ± SD for continuous data, and a comparison between different time points was done by analysis of one-way repeated measures test. $P < 0.05$ was considered as statistically significant and $P < 0.001$ was considered highly statistically significant.

RESULTS

Clinical observations

All treated sites exhibited favorable clinical healing with no suppuration or abscess formation [Figures 2f and 3]. Most of the sites exhibited favorable clinical healing; the most common complications were persistent edematous and inflamed gingivae beyond 1 week ($n = 3$), development of residual periodontal pockets ($n = 2$), and no reduction in RDs ($n = 2$). Table 1 summarizes the baseline data, effects of the intervention at 3 and 6 months, and the reported complications. In all the participants, there was an uneventful resolution of inflammation and restoration of normal architecture.



Figure 2: Preoperative view of the site (a) a full-thickness mucoperiosteal flap preserving the buccal interdental papillae was raised; (b) de-epithelization of the papillae was done; the recombinant human fibroblast growth factor-2-impregnated membrane was placed upon the recession site (c) and was stabilized by suturing it to the lingual papillae (d); the flap was coronally advanced as far as possible to cover the membrane (e) and was sutured to the buccal interdental papillae; most of the sites exhibited favorable clinical healing; the most common complications were persistent edematous and inflamed gingivae beyond 1 week (f)

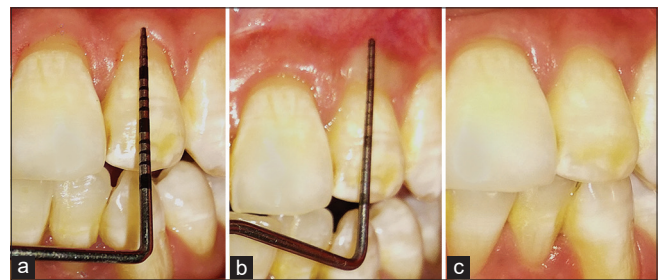


Figure 3: Observable outcomes from the procedure. Sites with gingival recession (a; upper right lateral incisor as an example) after therapy with recombinant human fibroblast growth factor-2-impregnated membranes showed the development of keratinized tissue over the treated sites (b; typical blanching on pressure) by 3 months. Closure of the recession almost to the level of the CEJ was seen at 6 months in most of the cases (c)

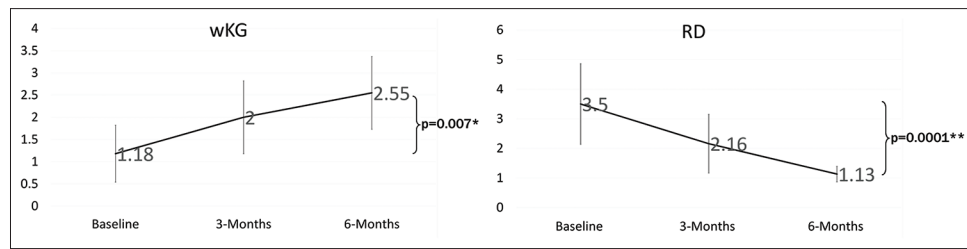


Figure 4: Comparisons at different time frames of the changes in width of keratinized gingiva and recession depth. **Highly significant ($P \leq 0.001$), *Significant ($P \leq 0.05$), wKG – width of keratinized gingiva

Table 1: Participant characteristics at different time intervals with associated complications (if present)

Participant number	Sites	wKG			RD			PD			Complications (if any)
		0 month	3 months	6 months	0 month	3 months	6 months	0 month	3 months	6 months	
1	22	1	2	3	4	2	1	1	2	1	Edematous and inflamed gingivae at the recipient site during suture removal
	23	1	2	2	4	2	1	1	2	1	
	24	1	2	2	3	2	1	1	1	1	
2	15	1	2	2	4	2	4	2	2	1	No improvement in recession depth
	12	1	2	2	4	3	1	1	2	1	
3	14	1	2	3	4	2	1	1	1	1	None
	41	2	2	3	3	2	1	1	2	1	
4	43	1	2	2	3	2	2	1	2	1	None
	32	1	2	3	3	2	1	1	2	1	
5	13	1	2	3	4	5	4	1	2	1	No improvement in recession depth. Mobile tissue seen
	21	2	2	1	3	2	1	2	2	1	
	22	2	2	2	3	2	1	1	2	1	
6	12	1	2	3	4	2	1	1	1	1	None
	14	1	2	2	3	3	4	4	1	1	
7	11	1	2	2	3	2	2	1	1	1	None
	34	1	2	2	3	2	1	1	1	1	
8	21	1	2	2	4	2	1	1	2	1	None
	22	1	2	3	4	2	1	1	2	1	
9	13	1	2	2	3	2	1	1	1	1	Development of residual periodontal pocket
	14	1	2	3	3	2	1	1	3	5	
10	21	2	2	2	4	3	1	1	2	1	None
	22	1	2	3	3	2	1	2	2	1	
11	32	1	L	L	4	L	L	1	L	L	None
	11	2	2	2	4	2	1	1	2	1	
12	22	1	2	2	4	2	1	1	1	1	Edematous and inflamed gingivae at the recipient site during suture removal
	31	1	2	3	3	2	1	3	1	1	
13	14	1	2	2	3	2	1	1	1	1	None
	21	2	2	2	4	2	4	1	2	4	
14	11	1	2	L	3	2	L	4	1	L	Development of residual periodontal pocket
	14	2	2	2	4	3	1	1	2	1	
15	12	1	2	3	3	2	1	1	1	1	None
	12	1	2	3	3	2	1	1	1	1	
16	11	1	2	2	4	2	1	1	2	1	Edematous and inflamed gingivae at the recipient site during suture removal. 41 was extracted because of trauma
	41	1	2	L	3	2	L	1	1	L	
17	23	1	2	2	3	2	1	4	2	1	None
	23	1	2	2	3	2	1	4	2	1	

wKG – Width of keratinized gingiva; RD – Recession depth; PD – Probing depth, L – Lost to follow-up

Intragroup comparisons

The wKG at baseline, 3 months, and 6 months was 1.18 ± 0.64 , 2.00 ± 0.82 , and 2.55 ± 0.85 mm, respectively. A significant increase ($P = 0.007$) in the wKG was noted from baseline to 6 months. The RD at baseline, 3 months, and 6 months was 3.50 ± 1.36 , 2.16 ± 0.99 , and 1.13 ± 0.26 mm, respectively. A highly significant decrease ($P = 0.0001$) in RD was noted from baseline to 6 months. The PD at baseline, 3 months, and 6 months was 1.47 ± 0.32 , 1.42 ± 0.48 , and 1.39 ± 0.08 mm, respectively [Figure 4]. There was no significant change in PD from baseline to 6 months ($P = 0.02$).

DISCUSSION

This study was designed as a single-arm trial to obtain preliminary data on the efficacy of collagen membranes impregnated with rhFGF-2 in the treatment of Miller’s Class I and II gingival recessions. rhFGF-2 stimulates the proliferation and migration of mesenchymal cells which later differentiate into cementoblasts, osteoblasts, and collagen-forming cells.^[14-17] Recently, a large-scale multicenter randomized clinical trial reported that the application of FGF-2 was efficacious in the regeneration of human periodontal tissue.^[18] Collagen-based biomaterials are commonly used as delivery vehicles for

protein drugs, including FGF-2, because they can form a stable polyanionic complex with FGF-2.^[19] Similarly, in the present trial, cross-linked collagen membrane was utilized as a carrier for rhFGF-2.

Studies on rhFGF-2 have focused on hard-tissue regeneration, where results have been weighed in terms of bone and cementum regeneration.^[10,14-19] Whereas, the present study evaluated the efficacy of rhFGF-2 on soft-tissue regeneration in sites with Miller's Class I and II gingival recessions. There is a paucity of data regarding the use of FGF-2 in root coverage. Cha *et al.*^[11] investigated the effect of FGF-2 in combination with porcine collagen matrix for coverage of root recession defects in dogs and observed that FGF-2 showed a higher amount of root coverage at 4 weeks and over 80% of mean root coverage could be achieved in 16 weeks.^[12] This is in agreement with the present study where a statistically significant amount of root coverage as evidenced by an increase in wKG and a decrease in RD was achieved by 3 months and remained stable until the end of the study period.

A study by Ishii *et al.*^[12] on the effect of FGF-2 and beta-tricalcium phosphate (β -TCP) on root coverage in dogs raised questions on the limited soft-tissue regeneration seen during the trial. Enhanced bone and cementum formation were observed in this study, which, however, could not translate into satisfactory root coverage;^[11] histological evaluation was not a part of our study design, and a comparison of our results with the above study is not possible. Contrary to the above, clinically, rhFGF2 impregnated in membrane form has shown an adequate gain in wKG from baseline to 6 months. The results are similar to the findings of Shujaa Addin *et al.*^[1] who evaluated rhFGF-2 in a gelatin/ β -TCP sponge in canine-gingival recession defects with an 8-week biopsy interval. Gelatin/ β -TCP/rhFGF-2 sites exhibited more tissue regeneration, characterized by larger amounts of new bone and new cementum when compared to gelatin/ β -TCP sites. In this study, complete root coverage has been observed in 8 weeks,^[1] a finding that was seen in this study as well through a significant increase in wKG and a higher amount of root coverage with rhFGF-2 membrane from baseline to 12 weeks/3 months. The resolution of factors implicated in gingival recession may also have contributed to these positive findings as well: (1) the absence of calculus, restorations, or necrotic cementum on the root surface;^[6,13] (2) the increased keratinized gingiva brought because of the healing effects of rhFGF-2,^[1,5,8,10] and (3) exposure of biomaterial could be a risk factor for its low predictability; however, we feel that the biological behavior of the material effectively counteracts this effect.^[14-17]

Healing was largely uneventful; only four participants showed residual periodontal pockets and no reduction in RDs at the end of the study period. rhFGF-2 initiates soft-tissue healing by periosteum formation, local neovascularization, and hypercellularity^[1] and promotes new attachment to the root surface by cementum formation,^[1,11,12,14-19] all of which contribute to adequate soft-tissue regeneration and root coverage.^[1,6-8,11,12] Edematous and inflamed gingivae beyond 1 week were the most common side effect seen; this may be explained by increased vascularity and tissue cellularity because of rhFGF-2.^[1]

This study has some limitations worth noting. Rather than a single-arm trial, an active-controlled trial comparing rhFGF-2 with an existing "gold standard" protocols such as mucogingival procedures with or without growth factor additives would have validated the efficacy of the material better. The study design was an open-label single-arm trial as our primary aim was to demonstrate the clinical impact of rhFGF-2-impregnated absorbable collagen membrane in the treatment of gingival recession defects. No measurement of gingival thickness nor any form of histologic evaluation was done; hence, the microscopic behavior of rhFGF-2 on the recession site remains unknown though previous studies have established *de novo* cementum and collagen formation in transplanted sites.^[1,11,12,14-18]

CONCLUSION

This trial showed that sites treated with rhFGF-2-impregnated absorbable collagen membrane showed a significant improvement in measures of gingival recession. The prospect of harnessing the potential of rhFGF-2 to influence periodontal wound healing in different surgical procedures is an exciting possibility that deserves further study.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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