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Concentrated growth factors in the treatment of adjacent multiple gingival recessions: A split-mouth randomized control trial

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ABSTRACT

Aims and Objectives: The aim of this study was to determine the clinical effect of Concentrated Growth Factor (CGF) in combination with Coronally Advanced Flap (CAF) compared to CAF alone in the treatment of adjacent multiple Miller's Class I GRs.

Materials and Methods: A total of 10 patients with Class I Miller's recession were randomly assigned by a coin toss method as Group I which received Coronally Advanced Flap alone (CAF) and Group II which received Coronally Advanced Flap (CAF) with Concentrated Growth Factor (CGF). The clinical parameters such as, Recession Height (RH), Recession Width (RW), Probing Pocket Depth (PPD), Clinical Attachment Level (CAL), Keratinized Tissue Width (KTW) and Thickness of Keratinized Tissue (TKT) were measured at baseline and 3 months postoperatively. The Mean Root Coverage (MRC), Complete Root Coverage (CRC) and Root coverage Esthetic Score (RES) were measured after 3 months postoperatively.

Results: Statistically significant difference in RH, RW, CAL, KTW, TKT, MRC and RES were observed on comparison of Group I and Group II at 3 months. On comparison, of Group I and Group II, no statistically significant difference was seen in PPD and CRC between the groups at 3 months.

Conclusion: The present study indicates that CAF with a CGF proved to be superior. The KTW and TKT augmentation might improve the long-term predictability of the root coverage procedures, hence the use of CGF combined with CAF, diminishes post-surgical relapse and thus providing long-term stability.

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1. Introduction

Periodontitis belong to the group of complex inflammatory diseases in humans. In this context, the word complex not only describes the fact that there are multiple clinical symptoms that account for the disease, but also explains the multiple factors that lead to and influence periodontal inflammation. The interaction between bacteria present in the plaque and immune response of the host results in matrix degradation, bone resorption, and downgrowth of the epithelium, resulting in periodontal pockets, gingival

recession, or a combination of both, thereby leading to periodontal diseases (Hangorsky U et al 1980).¹ The common etiologic factors for gingival recession are the local factors, periodontal disease, mechanical forces, iatrogenic factors and anatomical factors. (Susin C et al 2004).²

Gingival recession is the apical displacement of gingival margin from the cemento enamel junction. The Gingival Recessions (GRs) have been successfully treated by several periodontal plastic surgery procedures. The ultimate goal of these plastic periodontal surgical procedures is the coverage of exposed root surface and an optimal aesthetic outcome (Aroca et al. 2009).³

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Among the different types of procedures used, Coronally Advanced Flap (CAF) is the most frequent approach, and when combined with a Connective Tissue Graft (CTG) is accepted as a gold standard therapy (Graziani et al. 2014).⁴ Inductive tissue regeneration through recent advances in cellular biology occurs with the help of growth factors. Thus the three key cellular events in tissue repair are mitogenesis, migration and matrix synthesis and remodelling (Takata T. et al. 2015).⁵

Concentrated Growth Factors (CGF) was first developed by Sacco (Sohn et al. 2011).⁶ CGF is produced by the centrifugation of venous blood. However, a different centrifugation speed permits the isolation of much larger, denser and richer GFs in fibrin matrix from CGF. This fibrin clot has a high cohesion because of the agglutination of fibrinogen, factor XIII and thrombin. Thus CGFs' placement together with CAF may enhance the healing of soft tissues. Therefore, the aim of this study was to determine the clinical effect of CGF in combination with CAF compared to CAF alone in the treatment of adjacent multiple Miller Class I GRs.

2. Materials and Methods

The study sample was selected from the patient pool of the Department of Periodontics, Thai Moogambigai Dental College, Chennai. This study is designed as a split mouth-randomized controlled trial comparing two surgical protocols for the treatment of gingival recession: Coronally Advanced Flap (CAF) alone versus Coronally Advanced Flap (CAF) with Concentrated Growth Factor (CGF). Bilateral Miller's Class I Gingival Recession (GR) with 60 sites among 10 patients in the maxillary arch and the age between 18 to 50 years were selected. Recessions were randomly treated according to a split-mouth design by means of CAF with CGF or CAF alone. Clinical outcomes were evaluated at baseline and 3 months after surgery.

Patients were enrolled on the basis of following inclusion criteria: Age > 18 years, Presence of bilateral buccal Miller's Class I GR involving ≥ 2 teeth in the maxillary incisors, canine or premolar, Gingival Recession Depth (RD) of ≥ 2 mm, Probing Pocket Depth (PPD) of < 3 mm and Gingival Thickness (GT) ≥ 1 mm, Keratinized Gingiva Width (KGW) ≥ 2 mm, Presence of identifiable Cemento-Enamel Junction (CEJ), Controlled periodontal inflammation. Patients who had systemic problems that would contraindicate for periodontal surgery, Smokers, Usage of medications known to interfere with healing and to cause gingival enlargement, Recession defects associated with demineralization, deep abrasion, non-vital teeth, presence of caries or restorations, Previous surgery in the defected area within the past 1 year, Pregnant or lactating females were excluded from this trial.

3. Presurgical Protocol

The patients were informed about the type of treatment to be rendered and agreed to the study protocol. Informed consent was obtained from the patients, prior to the treatment. The protocol of the study was approved by the ethical committee of the institution. Each patient was prepared for surgery with an initial phase of therapy, which included scaling and root planing, oral hygiene instructions and occlusal adjustment. On completion of initial examination and through phase I therapy, the selected sites were assigned to either Group I or Group II by coin toss method. Following clinical parameters (Newman MG et al. 2013)⁷ were recorded at all-time intervals pre and postsurgery: Recession Height (RH): The distance between Cemento Enamel Junction (CEJ) to the most apical point of the Gingival Margin (GM), Recession Width (RW): The distance between the mesial and distal border of the recession, measured at the CEJ, Probing Pocket Depth (PPD): It is the distance between the base of the pocket and the gingival margin measured to the nearest millimetre at the gingival margin. Six sites (mesio-buccal, mid buccal, distobuccal, mesiolingual, midlingual and distolingual) of each tooth were taken, Clinical Attachment Level (CAL): The distance between the base of the pocket and a fixed point on the crown such as CEJ, Keratinized Tissue Width (KTW): Keratinized gingiva includes the marginal gingiva and the attached gingiva. It is calculated by measuring the distance between the most apical point of the gingival margin and mucogingival junction, Thickness of the Keratinized Tissue (TKT): Transgingival probing: Measured at a mid – point location between the gingival margin and mucogingival junction (3 mm from gingival margin) using an endodontic spreader. The spreader was pierced, perpendicular to the mucosal surface with light pressure through the soft tissue till hard surface was felt. Silicone disk stop was then placed in tight contact with external surface of soft tissue. The penetration depth was measured after careful removal of the spreader, Mean Root Coverage (MRC) (Naik et al 2013)⁸ was calculated according to the following formula.

$$\frac{\text{Post-operative recession depth}}{\text{Pre-operative recession depth}} \times 100\%$$

Complete Root Coverage (CRC) was calculated from the marginal gingiva to cemento-enamel junction for each treated site in a postoperative visit, Root Coverage Esthetic Score (Cairo F et al 2009)⁹ The gingival response to a anterior esthetic evaluation is assessed by the Root Coverage Esthetic Score (RES) from clinical photography according to five variables are Gingival Margin level (GM), Marginal Tissue Contour (MTC), Soft Tissue Texture (STT), Muco-Gingival Junction (MGJ), Gingival Color (GC). The best esthetic score was 10. A score of 0 was assigned when the final gingival margin position was equal or apical to the previous recession depth i.e, failure of the root coverage

procedure) independent of color, the presence of scarring, the gingival margin, or MGJ. A score of 0 was also assigned when a partial or total loss of interproximal papilla (a black triangle) occurred after the treatment.

4. Surgical Protocol

4.1. CGF preparation

Intravenous blood was collected in two 10-ml glass-coated plastic tubes without anticoagulant solutions. These tubes were then immediately centrifuged with a CGF centrifuge machine (Medifuge, Silfradentsr, S. Sofia, Italy) using a program with the following characteristics: 30'' acceleration, 2' 2700 r.p.m., 4' 2400 r.p.m., 4' 2700 r.p.m., 3' 3000 r.p.m., and 36'' deceleration and stop. At the end of the centrifugation there were four blood fractions: the upper serum layer, the second buffy coat layer, the third GF and unipotent stem cell layer (CGF) and the lower red blood cell layer (RBC). The CGF clot was removed from the tube and separated from the RBC by using surgical scissors. The CGF was squeezed that produces membranes at a constant thickness of 1 mm. The CGF membrane was immediately placed over the recession area.

5. Surgical Procedure

Patients were subjected to a periodontal full mouth professional prophylaxis. The appointment was scheduled 1 week prior to the surgical procedure. All surgeries were performed during a single surgical session. GR sites were randomly determined as either test or control site by tossing a coin immediately before the surgical procedure. After local anaesthesia using 2% Lignocaine HCL containing 1:80,000 epinephrine, the surgical procedure was carried out. The envelope type of CAF was proposed by Zucchelli and De Sanctis.¹⁰ The flap was raised with a split-full-split approach in the coronal–apical direction; the surgical papillae were elevated split thickness, keeping the blade parallel to the long axis of the teeth. This split elevation terminated at the level of an imaginary line connecting the probeable sulcular areas of the two adjacent recessions. Gingival tissue apical to the root exposures eliminated with the blade kept parallel to the external mucosal surface. During coronal advancement, each surgical papilla rotated toward the periphery of the flap and finally resided at the center of the interproximal area (anatomic papilla). Flap mobilization was considered adequate when the marginal portion of the flap was able to passively reach a level coronal to the CEJ at every tooth in the surgical area and when the surgical papillae covered the corresponding anatomic papillae. The flap should be stable in its final position without the sutures. All incisions were made using a Bard Parker-15 blade. The exposed root surfaces were planed using curettes (Gracey curettes; Hu Friedy, Chicago, IL, USA) to remove plaque, calculus and soft tooth structure.

No further root conditioning, mechanical or chemical, was performed. These procedures were the same in both of the groups. The CGF membrane was placed over the defect and extended apically beyond the apical base of recession defects by ≥ 3 mm in the test group. The flap was coronally positioned over the membrane to completely cover the CEJ and sutured with interrupted sling suture using 4.0 silk in the test group. The same procedure was performed without CGF membrane in the control group. Stabilization of blood clot was obtained with a gentle pressure for 3 min (Aroca et al. 2009, Eren & Atilla 2014).^{3,11}

5.1. Post-operative instructions and follow-up

The patients were informed to avoid brushing at the surgical site for 2 weeks. During this period plaque control was obtained by the use of 0.12% chlorhexidine solution twice daily and to consume a soft food diet for 2 weeks. Oral hygiene instructions were provided at each post-operative visit. Systemic antibiotics and analgesics were prescribed for 7 days post surgically (Amoxicillin, 500mg, tid, Ibuprofen, 400 mg, tid). Sutures were removed after 10 days, and patients were seen after 3 months. The clinical parameters were measured at baseline and 3 months postoperatively for both control and test group and compared.

6. Results

The clinical parameters which were assessed at baseline and 3 months were recession height, recession width, probing pocket depth, clinical attachment level and height of keratinized tissue. The other clinical parameters which were assessed at 3 months were thickness of keratinized tissue by using trans-gingival probing and root coverage esthetic score. The data was statistically analyzed to find the mean, standard deviation and tests of significance of mean values for the various parameters. Data was entered in Microsoft excel spread sheet and analyzed using SPSS software (version 21).

6.1. Clinical outcome

Table 1 Shows the comparison of mean value of Recession Height & Recession Width in Group I and Group II at baseline and 3 months. At 3rd month the mean value was in Group I and Group II which was statistically significant ($P=0.004$) and ($P=0.019$). The mean values of Probing Pocket Depth were not statistically significant at baseline ($P=0.987$) and 3 months ($P=0.863$). The mean value of Clinical Attachment Level at 3rd month was in Group I and in Group II which were statistically significant ($P=0.022$). The mean value of Keratinized Tissue Width at 3rd month was 3.03 ± 0.964 in Group I and 3.83 ± 0.912 in Group II, which was statistically significant ($P=0.001$). The mean value of Thickness of the Keratinized Tissue in Group I and

Table 1: Descriptivestatistics of clinical parameters between group I and group II at 3 months

Clinical Parameters	Time intervals	Group I	Group II	P- value
RH	Baseline	2.83±0.592	2.93±0.739	0.616
	3 months	1.43±0.626	0.93±0.639	0.004
RW	Baseline	2.63±0.556	2.70±0.702	0.837
	3 months	1.73±0.907	1.13±0.819	0.019
PPD	Baseline	3.30±0.836	1.53±0.507	0.987
	3 months	1.90±0.803	1.86±0.819	0.863
CAL	Baseline	4.13±0.836	4.23±0.773	0.823
	3 months	3.30±0.836	2.73±0.868	0.022
KTW	Baseline	1.96±0.668	1.93±0.583	0.851
	3 months	3.03±0.964	3.83±0.912	0.001
TKT (Trans-gingival probing)	Baseline	1.33±0.479	1.36±0.490	0.788
	3 months	2.03±0.413	3.06±0.520	0.001

Data are expressed as the mean ± standard deviation (Mean Rank). Values in bold are statistically significant ($p < 0.05$), Mann Whitney U Test

Table 2: Comparison of mean root coverage(MRC) between Group I and Group II at 3 months

Groups	MRC	Mean difference	Z	P value
Groups I	49.16±23.40	-18.61	-2.998	0.003
Graoup II	67.77±20.84			

Data are expressed as the mean ± standard deviation (Mean Rank). Values in bold are statistically significant ($p < 0.05$), Mann Whitney U Test

Table 3: omparison of complete root coverage(CRC) between Group I and Group II at 3 months

Groups	Group I	N	CRV		Total	Chi Square	P value
			Complete root coverage	Not complete			
	Group I	N	2	28	30	3.268	0.071
	Group II	N	7	23	30		
Total		N	9	51	60		
		%	15.0%	85.0%	100.0%		

Chi-square test.

Table 4: Comparison of root coverageesthetic score (RES) between the Group I and Group II at 3 months

Group	Res	Mean difference	Z	P value
Group I	Mean±S.D			
	5.55±1.46	2.86	-5.793	0.001
Group II	8.40±1.30			

Data are expressed as the mean ± standard deviation (Mean Rank). Values in bold are statistically significant ($p < 0.05$), Mann Whitney U Test

Group II were and at 3 months were statistically significant ($P=0.001$).

Table 2 Shows the comparison of Mean Root Coverage between Group I and Group II at 3 months. In Group I and Group II the mean values were respectively which were statistically significant ($P=0.003$).

Table 3 Shows the comparison of Complete Root Coverage between the Group I and Group II at 3 months. The Complete Root Coverage value in Group I was 6.7% and Group II was 23.3%, which were not statistically significant ($P=0.071$).

Table 4 depicts the comparison of mean value of Root CoverageEsthetic score between Group I and Group II at 3 months. The mean at 3rd month in Group I and Group II, which were statistically significant ($P=0.001$).

7. Discussion

The term “coronally advanced flap” was coined by Pini-Prato et al. in 1999,¹² to better reflect the procedure and CAF has been shown to predictably achieve root coverage. The average root coverage achieved with this technique ranges from 75% to 82.7%, with 24% to 95% of sites achieving complete root coverage. Among the

different types of procedures used, Coronally Advanced Flap (CAF) is the most frequent approach, and when combined with a Connective Tissue Graft (CTG) is accepted as a gold standard therapy (Aroca et al. 2009, Graziani et al. 2014).^{3,4} Zucchelli et al.¹⁰ suggested an envelope incision for maintaining lateral flap vascularization and a better postoperative course. However, both incisions have been found to be effective in RC. The flap was elevated using partial–full–partial thickness in the manner described by Zucchelli et al.¹⁰ The aim of using this technique was to preserve the blood supply coming from the adjacent periosteum, as well as the flap portion covering the root surface and the CGF.

Concentrated Growth Factors (CGF) first developed by Sacco (2006),¹³ is a relatively new technology within the area of regenerative medicine. CGF is an advanced third generation platelet concentrate, CGF is a fibrin rich organic matrix which contains growth factors, platelets, leukocytes and CD34+ stem cells which help in the process of regeneration and also has immunological cells that are effective in regulating inflammation and minimizing the risk of infection.

Most of the studies in this field have investigated the effects of PRP and PRF on RC procedure. The effects of autogenous PCs on clinical outcomes of the surgical treatment of periodontal diseases were evaluated in a systematic review (Del Fabbro et al. 2011).¹⁴ They concluded that PCs did not have significant benefit for the treatment of GR. However, in another systematic review, Luo et al. (2015)¹⁵ concluded that the additional use of PCs might exert a positive effect in the treatment of GR and wound healing. Since then, there were an increasing number of researches about the application of PCs in the treatment of GR.

Recently, the use of CGF, as an alternative PC, has been reported with limited data (Sohn et al. 2009)¹⁶ This fibrin clot has a high cohesion because of the agglutination of fibrinogen, factor XIII and thrombin. This provides protection from plasmin degradation, resulting in higher fibrin tensile strength and stability (Rodella et al. 2011, Kim et al. 2014).¹³⁻¹⁷

Platelet concentrates have been used for repair of intra-bony defects (Camargo et al. 2002, Thorat et al. 2011).^{18,19} furcation defects (Lekovic et al. 2003, Sambhav et al. 2014)^{18,20} and sinus augmentation (Froum et al. 2002, Tajima et al. 2013)^{21,22} as promoters of tissue regeneration (Anilkumar et al. 2009).²³ All these procedures have demonstrated new bone formation and bone healing. Recently, investigators reported the use of CGF in the re-construction of the bone defects. Therefore, this study hypothesized that CGF placement together with CAF may enhance the healing of soft tissues.

On comparison of mean recession height between Group I and Group II at baseline did not show

statistically significant difference, but significant difference was observed at 3 months in our study similar to the results obtained by Dogan et al (2015),²⁴ Cordioli G et al. (2001).²⁵ Growth factors present in the CGF which influenced the gingival and periodontal fibroblasts proliferation. According to Wilderman and Wentz,²⁶ the healing of a pedicle flap on the denuded root surface includes the adaptation stage, the proliferation stage, the attachment stage, and finally, the maturation stage.

On comparison of mean recession width between Group I and Group II at baseline, it did not show statistically significant difference, but significant difference was observed at 3 months in our study similar to the results obtained by Trombelli L (1999),²⁷ Chambrone LA & Chambrone L (2006).²⁸ This finding may be due to the fact that during the exposure of the root surface to CGF, insoluble fibrin networks form providing a scaffold for cell migration, proliferation and upregulating collagen (type I) synthesis in extracellular matrix. Platelets undergo a change in shape (becoming flatter, forming pseudopods), and aggregates on the wound surface. Degranulation follows ensuring the release of various growth factors. Growth factors released from platelets elicit their biologic effects by binding to cell-surface receptors.

There was no significant difference in the probing pocket depth in Group I and Group II at baseline and 3 months ($P=0.987$, $P=0.863$ Table 3), which were comparable to the results obtained by Dogan et al (2015)²⁴ and Aroca et al. (2009),³ there was no significant gain in the clinical attachment levels between the groups, but significant difference was observed at 3 months. The results were found to be comparable with the results by Dogan et al. 2015,²⁴ Cordioli G et al 2001.²⁵

In the present study, shallow PPD and improved CAL values were observed at the 3-month evaluation, the healing is characterized by initial adaptation of the graft to the recipient bed by a fibrin layer with epithelial downgrowth of the flap tissue. Later there is proliferation and invasion of the fibrin into the connective tissue along with cementum formation in the root surface by the cementoblasts. This phase is followed by attachment of the fibres to the cementum and increase in the number of collagen fibers (Wilderman MN and Wentz FM 1965).²⁶ This phenomenon justifies the process of reduction of probing depth with increased clinical attachment level.

The mean value of keratinized tissue width shows no significant difference was found between the groups at baseline and significant difference was found at 3 months which was correlating with the results obtained by Dogan et al. (2015),²⁴ Padma et al.(2013)²⁹ and Cheung & Griffin (2004)³⁰ found an increase in keratinized gingival width for the CAF alone and CAF-PC grafts, respectively. There was significant increase in the thickness of keratinized tissue gain in Group I and Group II from baseline ($P=0.001$, to

3 months ($P=0.001$, similar to the study by Dogan et al. (2015),²⁴ Cheung & Griffin (2004)³⁰ who found an increase in TKT for the CAF alone and CAF-PC grafts, respectively.

Cheung et al.³⁰ reported that the keratinized tissue width and gingival thickness gain in Platelet concentrate group was similar to Subepithelial Connective Tissue Graft group and suggested that this could be due to the influence of Growth Factors on gingival fibroblasts. Platelet concentrate will stimulate the gingival connective tissue with key matrix proteins for cell migration (fibronectin, vitronectin, and thrombospondin-1). It maintains the flap in a newly repositioned and stable position, enhances neoangiogenesis, reduces necrosis and shrinkage of the flap, and, thus, guarantees maximal root covering. Because of all these properties, it aids in formation of good thickness of keratinized tissue around the treated site.

The presence of keratinized gingiva is an important factor for the maintenance of gingival health and prevention of periodontal disease progression. In this study, the higher increase in KGW and TKT in the test group may be explained by biology of CGF, which contains much larger, denser and richer in GFs fibrin matrix (Sohn et al. 2009, Rodella et al. 2011).^{13,16}

The percentage of mean root coverage was significantly higher in Group II compared to Group I ($P=0.003$). The percentage of complete root coverage at 3rd month was 6.7% in Group I and 23.3% in Group II. The mean values were not statistically significant ($P=0.071$). Complete Root Coverage depicts the recovery from hypersensitivity and aesthetic factors associated with recession. Significant creeping attachment can be observed when one of the platelet concentrates is interposed under the flap, but the length of time for this observation may vary among mucogingival techniques.

Further in the present study, the mean value of root coverage esthetic score at 3rd month were statistically significant ($P=0.001$, Table 9). The results were consistent with that obtained by Salhi L et al (2014).³¹ One of the reasons to treat gingival recession, is to achieve better aesthetics. However, aesthetic outcomes after surgical root coverage procedures are rarely evaluated in the literature. Since the soft tissue maturity is considered stable after a period of 3 to 6 months post-surgically according to Rocuzzo M et al (2002)³² and Cairo F et al. (2009),⁹ the aesthetic outcomes of the present study were evaluated after 3 months. CGF membrane was used per surgical site, and the key principle for use of these membranes are thin fibrin scaffolds and might be quickly resorbed in the gingival environment where vascularization is very efficient; to influence the long-term stability of the stimulated tissue, the fibrin-based cicatricial matrix must be thick and strong, particularly for the covering of multiple adjacent recessions. CGF is also a healing and interposition biomaterial.

This study with time period of three months have indicated that the treatment of gingival recession by

coronally advanced flap alone and along with concentrated growth factor resulted in reduction in recession height, recession width and gain in clinical attachment and keratinized tissue height, with an increase in the gingival thickness, comparatively more in CGF with coronally advanced flap. The obvious advantage of CGF are autologous, cost effective regenerative material. The peculiar quality of CGF which is again proved in this study is the thickness of the marginal tissue which provides a great advantage in preventing further recession in future and in maintenance of the root coverage obtained.

The study must be interpreted with due consideration to the following limitations such as relatively small sample size and shorter evaluation period of three months. A well designed histological, human and animal studies with long term follow up is needed to confirm the findings.

8. Conclusion

Treatment of gingival recession has become an important therapeutic and esthetic issue for the contemporary periodontal practice due to an increasing public demand for cosmetic dentistry... Careful case selection and surgical management are critical if a successful outcome is to be achieved. It is a known fact that the etiology of gingival recession is multifactorial and its appearance is always the result of more than one factor acting together. Therefore, the surgical techniques conducted should also take into consideration the elimination of the causative factors for better prognostic outcome. Further the usage of advanced generation of Platelet concentrates CGF, its high acceptance, affordability, lower acquisition and maintenance has set forth to newer dimensions in periodontics. This method will certainly aid clinicians in the planning and execution of a number of treatment procedures in dentistry with increased predictability.

Within the limitations of this study, it can be concluded that CAF with CGF was superior to CAF alone in providing a consistent reduction in the baseline recession height and width. The KGW and TKT augmentation might improve the long-term predictability of the root coverage procedures, hence the use of CGF combined with CAF, diminishes post-surgical relapse and thus providing long-term stability.

9. Source of Funding

None.

10. Conflict of Interest

None.

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