

Evaluation of Regenerative Potential of Locally Delivered Vitamin C along with Microneedling in the Treatment of Deficient Interdental Papilla: A Clinical Study

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ABSTRACT

Aim: To evaluate the efficacy of intrapapillary injections of vitamin C along with microneedling (MN) in enhancing deficient interdental papilla (IDP) in esthetic zones clinically.

Materials and methods: Fifteen patients diagnosed with black triangles according to Norland classification system for loss of papillary height were selected. After oral prophylaxis and baseline measurements of insufficient interdental papilla, the vitamin C injection was loaded in disposable syringe 30G × 1/2" needle. The needle was inserted at 45° angle, 2–3-mm apical to the involved papilla and all surrounding areas. Each involved papilla was injected with an amount till blanching was visible. After that, MN was done in the required area. This method was repeated for 5 times at 7 days intervals consecutively. The final clinical photographs and measurements were recorded after 7 days of last dose administered.

Results: There was a statistically significant difference in the mean IDP height observed over the time period ($p = 0.002$).

Conclusion: An overall healthy appearance of gingiva with a remarkable increase in papilla growth was observed.

Clinical Significance: Vitamin C injection along with MN can be considered as an alternative to invasive surgical procedures in reconstruction or regeneration of the missing IDP.

Keywords: Interdental Papilla, Microneedling, Periodontal therapy, Regeneration, Vitamin C.

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INTRODUCTION

The existence or non-appearance of the interproximal papilla or so called "black triangle disease" is of prodigious distress to patients. The loss of papilla not only cause cosmetic deformities but also leads to phonetic problems due to passage for the air or saliva through these tiny spaces between the teeth. Frequently, the loss of papilla is a consequence of periodontal disease because of gingival inflammation, attachment loss and interdental bone loss/resorption. Missing papillae can also result from periodontal surgical therapy, and improper oral hygiene practices including improper tooth brushing/interdental cleaning, that is, improper flossing and tooth pick use, anatomical variations such as abnormal shape of the teeth, and tooth position, microdontia, or missing teeth/spacing between the teeth. Reconstruction of the mislaid IDP is one of the most thought-provoking and least foreseeable problem. In the past quite a few cases, presentations have been circulated encompassing of countless surgical and prosthetic methods to re-erect lost papillae, such as papilla recontouring, papilla preservation, and papilla reconstruction.^{1–5} They have had limited success and have lacked certainty.^{6–11} There are few other case reports which discusses the non-surgical, methods of IDP correction. Becker et al.¹² used commercially available injectable hyaluronic acid gel for deficient IDP in a small area, the first report to shows that minimally invasive and predictable rebuilding of scarce IDP was conceivable.

Vitamin C is a proven anti-inflammatory agent.^{13,14} and also exerts a reducing and antioxidant effect, scavenges free radicals, and acts as an enzyme cofactor in cells.¹⁵ This nutrient is considered to be an important dietary oxidant for periodontal health,¹⁶ as it plays a crucial role in preventing and slowing the progression of

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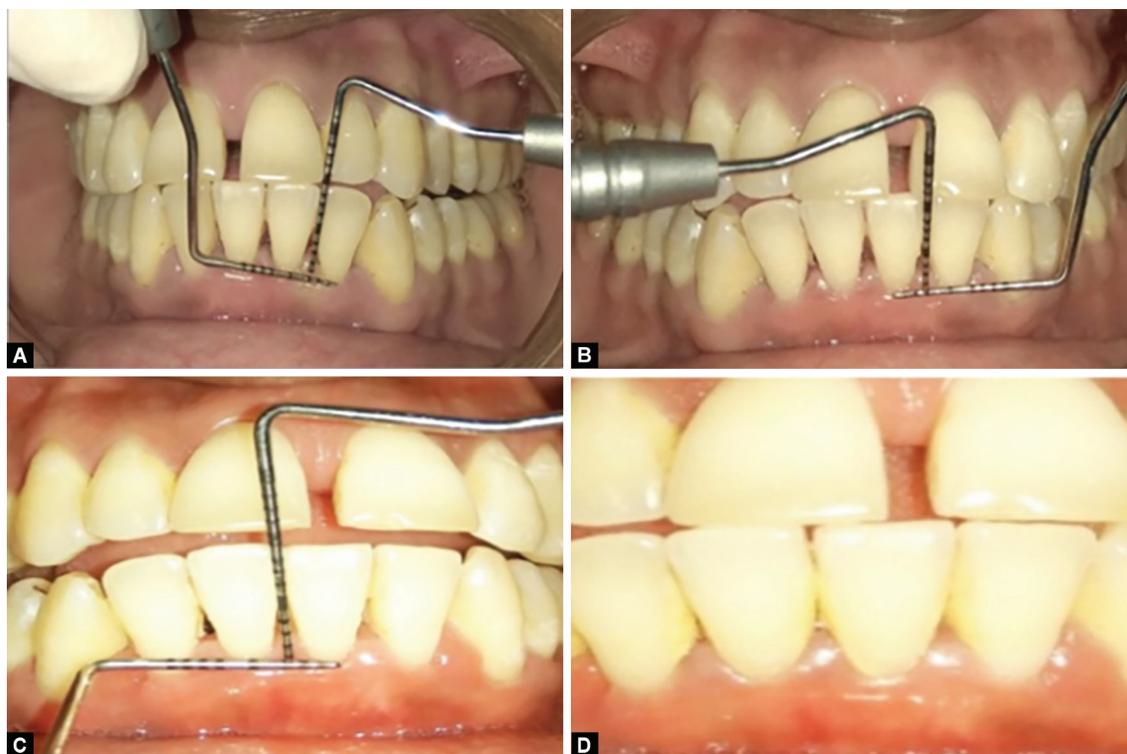
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periodontal disease by inducing the differentiation of periodontal ligament progenitor cells.¹⁷

Vitamin C has a very important role in collagen biosynthesis (collagen type I), as it helps in fibroblasts proliferation. It reduces the potentiality of scarring via inhibiting cross-linking of collagen fibers and fibrosis. It acts as a cofactor in hydroxyproline synthesis to produce collagen type IV and improves endothelial cell vitality and function.¹⁸

Microneedling is a newfangled negligibly offensive practice including superficial and skillful perforating of the skin by rolling with miniature fine needles. Over a petite period of time, it has expanded mass admiration and acceptance as it is a simple, inexpensive, and effective technique. Conventionally used as a collagen induction therapy for facial scars and skin reconstruction, it is



Figs 1A to D: Clinical measurements of IDP of tooth Nos. 41, 42, 31, and 32. (A) Baseline measurements of IDP, BTH/BTA with UNC 15 probes; (B) BTH/BTA measurements on first follow-up; (C) BTH/BTA measurements on second follow-up; (D) On final visit, complete IDP fill is observed. IDP, interdental papilla; BTH, black triangle height; BTA, black triangle area

also largely used now a days as a transdermal delivery system for therapeutic drugs and vaccines. The MN aids to distinct the cells instead of wounding through establishing microconduits which upsurges the skin's permeability and blood flow into the epidermis. This course facilitates the dispersion of topical medications across the stratum corneum layer. Besides, the growth factors are produced promoting the regeneration of collagen and elastin.¹⁹

Recently, in the field of periodontics, various studies have been conducted to check the effectiveness of MN along with injectable platelet rich fibrin (iPRF) on thin gingiva, proving its beneficial role in achieving improvement in gingival biotype. Till now, no study has been mentioned in the literature evaluating effectiveness of Vitamin C along with MN in regeneration of inadequate IDP. Therefore, this study is an elusive attempt to evaluate the efficacy of intrapapillary injections of Vitamin C^{20,21} along with MN in enhancing deficient IDP in esthetic zones clinically.

MATERIALS AND METHODS

Study Design

Patient Selection and Ethical Clearance

This study was conducted in the Department of Periodontics and Implant Dentistry, Hazaribag College of Dental Sciences and Hospital, Hazaribagh, Jharkhand, India, with approval from the institutional ethical board. All participants in the study were provided with an overview of the clinical trial and guidelines. Patients were recruited only after signing the written informed consent. A total sample population of 15 patients was selected for the study. Patients of age ranged 25–35 years, and with classes I and II IDP loss according to Norland et al. 1998,²² were included in the

study. The subjects excluded were alcoholic and smokers, pregnant women, systemic unhealthy patients, patients having history of trauma from occlusion, patients under any systemic medication that risks gingival enlargement, patients receiving orthodontic treatment and patients with implants, and endodontically treated teeth.

After the selection of study subjects, black triangles area (BTA) and black triangles height (BTH) were measured clinically with UNC 15 probe (Figs 1A to C), and photographic images were also maintained to compare pre- and postoperative results.

Patient Preparation

Each patient received a proper knowledge of the study protocols along with full-mouth scaling and root planing (SRP) using ultrasonic and hand instrumentation and received personalized oral hygiene instructions. Recruited patients were placed on 7 days maintenance and recalled for 06 consecutive scheduled appointments.

Non-surgical Procedure

After the oral prophylaxis and baseline measurements of insufficient IDP, the vitamin C injection (Lifestar Mankind Pharma Pvt. Ltd.), India was loaded in disposable syringe 30G × 1/2" needle. The needle was inserted at a 45° angle, 2–3 mm apical to the involved papilla and all surrounding areas (Fig. 2). A single-point injection technique was employed, and the bevel of the injection needle was applied sloping upward. Each involved papilla was injected with an amount till blanching was visible (which is approximately 1–1.5 mL).^{13,14} After that, MN was done in the required area with a lancet needle which is used for pricking the finger during blood sugar examination (Fig. 3). This method was repeated for 5 times at 7 days intervals

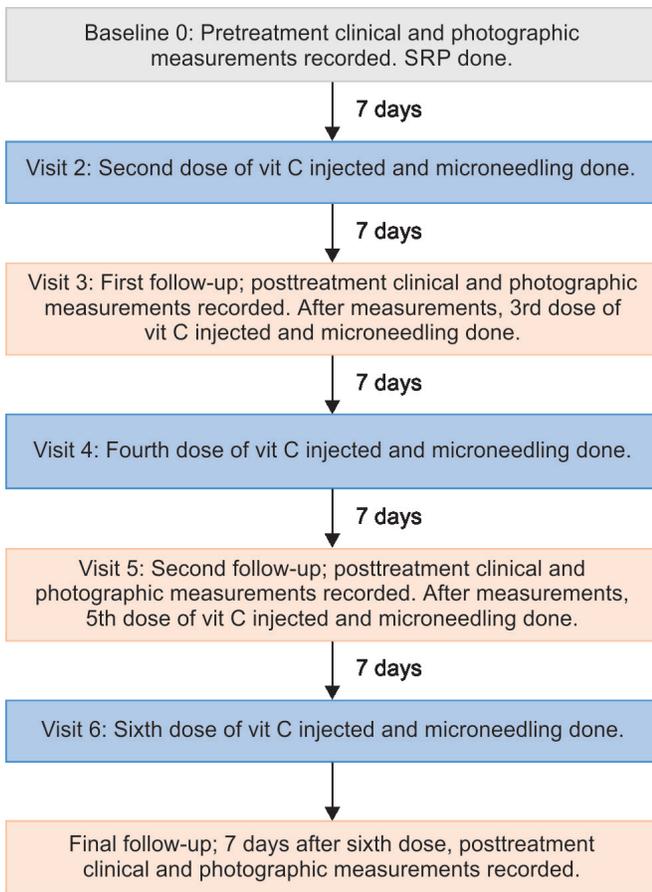


Fig. 2: The IDP vitamin C injections at tooth Nos. 41, 42, 31, and 32



Fig. 3: The IDP microneedling injections at tooth Nos. 41, 42, 31, and 32

Flowchart 1: Flowchart to elaborate study design



consecutively. The final clinical photographs and measurements were recorded after 7 days of last dose administered. Photographs were clicked at every visit up to 7 sequential visits (Flowchart 1).

Posttreatment Instructions

Post-treatment oral hygiene maintenance and care were educated to the patients. Use of floss and toothpick was discouraged during the study period.

Statistics were computed using IBM SPSS for Windows (version 25). A total of 15 samples were included in this study where mean and standard deviation was computed for the continuous variable and inferential statistics were computed using paired *t*-statistics, where comparison between individual follow-up was made.

RESULTS

Table 1 states the mean IDP height as presented for individual follow-up. A gradual increase in the mean height was noted over the period of time. The mean value of IDP height was 3.20 ± 0.274 mm; at the baseline, 3.46 ± 0.456; at the first follow-up, 3.76 ± 0.532 mm; and 4.10 ± 0.652 mm at the third follow-up, respectively. There was a statistically significant variation in the mean IDP height observed over the time period (*p* = 0.002).

Table 2 describes the comparison of the mean IDP height over the period of time. The mean increase of IDP height was 0.260 ± 0.261 mm from the baseline to the first follow-up, 0.560 ± 0.336 mm from the baseline to the second follow-up, and 0.900 ± 0.418 mm from the baseline to third follow-up, respectively. The mean increase of IDP height was 0.300 ± 0.122 mm from the first to the second follow-up, 0.640 ± 0.219 mm from the first to the third follow-up, and 0.340 ± 0.152 from the second to the third follow-up, respectively. A statistically significant difference was noted between the baseline and the second follow-up (*p* = 0.020), baseline and the

Table 1: The descriptive statistics for the IDP height at different intervals

	Mean	Standard deviation	Minimum	Maximum	Percentiles			p-value
					25th	50th (median)	75th	
Baseline	3.20	0.274	3	4	3.00	3.00	3.50	0.002*
First follow-up	3.46	0.456	3	4	3.00	3.50	3.90	
Second follow-up	3.76	0.532	3	5	3.25	3.80	4.25	
Third follow-up	4.10	0.652	4	5	3.50	4.00	4.75	

*Statistically significant

Table 2: Comparison of the mean IDP height as compared between intervals

	Paired differences						
	Mean	Standard deviation	Standard error mean	95% Confidence interval of the difference			p-value
				Lower	Upper	t	
Baseline: First follow-up	0.260	0.251	0.112	-0.572	0.052	-2.316	0.081
Baseline: Second follow-up	0.560	0.336	0.150	-0.977	-0.143	-3.725	0.020*
Baseline: Third follow-up	0.900	0.418	0.187	-1.419	-0.381	-4.811	0.009*
First to second follow-up	0.300	0.122	0.055	-0.452	-0.148	-5.477	0.005*
First to third follow-up	0.640	0.219	0.098	-0.912	-0.368	-6.532	0.003*
Second to third follow-up	0.340	0.152	0.068	-0.528	-0.152	-5.013	0.007*

*Statistically significant

third follow-up ($p = 0.009$), first and second follow-up ($p = 0.005$), first to third follow-up ($p = 0.003$), and also second to third follow-up (0.007). This study inferred that there was an increase in the overall IDP height over the period of time. A remarkable difference in the IDP height was noted from the baseline to the third follow-up along with improvement in gingival biotype (Fig. 1D).

DISCUSSION

Due to the higher potential antioxidant effects of vitamin C, there is considerable interest in whether higher than recommended levels of vitamin C are associated with better periodontal health or may support healing of periodontal tissues after specific procedures such as deep SRP, also known as “sanative therapy.”

As an antioxidant, vitamin C provides protection against oxidative stress-induced cellular damage by scavenging of reactive oxygen species, and it is used widely in beauty and cosmetic procedures. This study was conducted in the Department of Periodontics and Implant Dentistry, Hazaribag College of Dental Sciences and Hospital, Hazaribagh, Jharkhand, India. This study was conducted to evaluate the effects of vitamin C on IDP regeneration.²¹ It was shown that Vitamin C was effective in improving gingival texture, it gave a healthier appearance to the gingiva and was helpful in construction of papilla height mostly after second injection and MN; thus, we can say exogenous vitamin C could contribute to the maintenance of optimal collagenic density in the dermis and locally strengthen the collagen network.

In a similar study done by N Boyera et al.,²³ two *in vitro* models, designed to evaluate the effects of vitamin C on collagen biosynthesis and cross-linking at cellular and tissue levels. It was seen that vitamin C induced a dose-dependent increase in collagen type I deposits by normal human fibroblasts (NHF) cultured in monolayer, and enhanced extracellular matrix contraction by NHF in a lattice model, in a non-cytotoxic range of concentrations (103, 104, and 105 μ M). Vitamin C-phosphate (Vit C-P) and vitamin C-glucoside (Vit C-Glu), the two Vitamin C derivatives presenting higher chemical stability in aqueous solution, were also tested in this study, and showed similar biological properties, but with different potencies.

El-Mofty et al.²⁴ conducted a study to check the efficacy of intra-mucosal injection (mesotherapy) with topical gel as non-surgical approaches for handling gingival hyperpigmentation. Vitamin C mesotherapy revealed better and prompt effect than topical gel, and both techniques were not agonizing and esthetically substantial in managing gingival hyperpigmentation. Gingival melanin pigmentation causes esthetic apprehensions for noteworthy

number of patients. Inspecting non-surgical depigmentation techniques to decrease postoperative complications and patient discomfort, pain, and long curative period associated with invasive methods would be clinically significant.

A study conducted by Ozsagir et al.²⁵ and the inference drawn was that in individuals with thin periodontal phenotypes, standalone iPRF and iPRF with MN may have an influence in increasing gingival thickness (GT). The results suggest that application of iPRF and MN may be a first step of non-surgical method for increasing gingival thickness.²⁵

This improvement in gingival biotype and regeneration of IDP could be due to mechanism of collagen biosynthesis can be explained as L-ascorbic acid motivates procollagen synthesis in cultured human skin fibroblasts without appreciably altering non-collagen protein synthesis. The effect is unconnected to intracellular dilapidation of afresh synthesized procollagen. The cDNA probe hybridization was done levels of mRNA for pro α_1 (I), pro α_2 (I), and pro α_1 (III), which were seen elevated in the presence of ascorbic acid, whereas the level of mRNA for fibronectin is unchanged. Along with these, the levels of functional mRNA for procollagen increased in the presence of ascorbic acid. Thus, ascorbic acid seems to govern the countenance of three dissimilar procollagen genes, each of which is positioned on a distinct chromosome. Ascorbate deficiency may lead to a translational repression of procollagen synthesis of intracellularly accumulated procollagen. Ascorbic acid may dismiss this block by endorsing hydroxyproline formation and, therefore, secretion of procollagen from the cell. The increased level of procollagen mRNA under the influence of ascorbic acid may be secondary to increased synthesis of procollagen polypeptides; the control point may be gene transcription or mRNA degradation.²⁰

Our study has also shown positive results in regeneration of IDP using intrapapillary injections of vitamin C along with MN. There was improvement in gingival biotype when compared from baseline to last follow-up. Apart from intrapapillary injections, vitamin C can also be administered topically in a gel form or as a dentifrices²⁶ and locally into the subperiosteum.²⁷ In another study, a different mode of administration was discussed, ascorbic acid solution (ampoules 250 mg/mL) was applied for 5 minutes into the periodontal pocket by using a blunt-ended cannula after isolation of the area.²⁸

Hence, it should be considered as an excellent non-surgical method in regeneration of IDP loss in systemically healthy patients. Locally delivered vitamin C along with MN is a cost-effective,

non-invasive treatment modality with least side effects. Easy availability of the material with requirement of simple armamentarium makes it easy to implement in day-to-day practice.

Although there are no severe adverse effects of injectable vitamin C, multiple visits are required to achieve the desired results. Injections might be painful. Precautions may be needed in the following conditions: Vitamin C supplementation is contraindicated in blood disorders such as thalassemia, G6PD deficiency, sickle cell disease, hemochromatosis, and angioplasty (it may quicken the thickening of artery walls, a condition known as atherosclerosis). Diabetic patients should take vitamin C supplements with care as it raises blood sugar levels. Vitamin C should be used cautiously in oxalate nephropathy or nephrolithiasis as acidification by ascorbic acid increases the chances of precipitation of cysteine, urate, and oxalate stones.²⁹

CONCLUSION

This study inferred that there was an increase in the overall IDP height over the period of time. A difference in the IDP height was noticed after second dose in follow-up. An overall healthy appearance of gingiva, improvement of gingival biotype and a remarkable increase in papilla height were observed after completion of administration of last dose of injectable vitamin C along with MN. Hence, this protocol should be considered as a cost-effective, easy, and non-invasive method in esthetic corrections in IDP loss. However, the future long-term studies are required with a larger subject size to see its long-term effects.

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