ABSTRACT.

Increasing demands of patients for gingival esthetic and management of deficient papillae in the anterior region had stimulated minimally invasive techniques for papilla reconstruction with biological molecules.

Purpose: To evaluate the effect of hyaluronic gel injections in deficient papillae and monitor the results for a 6-months period.

Materials and methods: 57 class I and class II deficient papillae were included. 4 weeks after scaling and root planning hyaluronic acid gel was injected in the papillae. The injections were repeated in 20 days. Measurements of papilla height were done on the clinical photographs. The distance between the contact point (complete fill of interproximal space) to the most coronal level of the visible gingival margin was measured before treatment and on months 1, 3 and 6 and compared to baseline.

Results: There was a statistically significant change in the papilla level and fill of the interproximal space, ranging from 59% papilla gain on the first month, 72% to the third month and 77% on 6 months.

Conclusion: Hyaluronic acid gel is a reliable method for mini-invasive papilla augmentation, especially in class I and class II papilla deficiencies.

Keywords: deficient papilla, minimally invasive reconstruction, hyaluronic acid,

Facial aesthetics is especially associated with the smile zone and one of the main components for a beautiful smile is “pink” esthetics, with harmonious ratio parameters between teeth and gingiva. Presence of healthy interdental papilla, filling completely the interdental embrasure, is of key importance. The loss of papilla can lead to esthetic problems – so-called “black triangles”, which are prevalent among the adult population (38% of 119 cases) [1]. Black triangles lead also to phonetic problems (empty interdental space allows passage for the air or saliva) and lateral food impaction, leading to plaque deposition, gingival inflammation and subsequent additional soft tissue loss [2]. A study by Ziahosseini et al reported, that both patients and dentists consider black triangles of 3 mm to be unesthetic and less attractive [3]. Another study assessing 80 randomly selected patients’ perceptions of black triangles in terms of visible triangles and their severity showed that patients found the presence of black triangles the third most disliked aesthetic problem after caries and crown margins [4]. A unilateral papillary height reduction of 3 mm was easily detectable and the asymmetry considered unattractive [5].

The etiology of interdental papilla loss is multifactorial. The major causes include abnormal tooth shape, gingival biotype, diverging roots and root angulation, over-hanging margins of fillings and improper contours of prosthetic crowns; traumatic interproximal oral hygiene procedures, periodontal disease and loss of attachment, orthodontic treatment, flap surgery; spacing between teeth and loss of teeth [6, 7].

Nordland and Tarnow [8] proposed a classification system regarding the papillary height using three reference points, i.e., contact point, facial and apical extent of cementoenamel junction (CEJ), and interproximal extent of CEJ (iCEJ), and was classified into the following four classes:

Normal: The interdental papilla occupies the entire embrasure space apical to the interdental contact point/area (Fig.1 a).

Class I: The tip of the interdental papilla is located between the interdental contact point and the level of the CEJ on the proximal surface of the tooth (Fig.1 b).

Class II: The tip of the interdental papilla is located at or apical to the level of the CEJ on the proximal surface of the tooth but coronal to the level of the CEJ mid-buccally (Fig.1 c).

Class III: The tip of the interdental papilla is located at or apical to the level of the CEJ mid-buccally (Fig.1 d).
A crucial factor for loss of papilla and the possibility for is the distance between the interproximal contact point and alveolar bone crest. The study by Tarnow et al. defined “the rule of 5 mm” [9]. They found out, that when the vertical distance between the contact point and bone crest was d≥5 mm, the papilla is present in almost 100% of cases. When the distance is 6 mm, the papilla was present in 56% and when it is 7 mm or more – papilla is present only in 27% of the cases or less.

There are different approaches to treat lost papillae. Since the etiology is multifactorial, the patient may present with one or more etiologic factors, thus managing each patient requires an individual assessment and treatment plan. The non-surgical technique includes correction of traumatic oral hygiene procedures. Patients should be carefully instructed and controlled for their hygiene routine since this is an important preventive measure against papilla loss. The orthodontic approach includes closure of the interdental space. Closure of diastema and creation of a contact point between the adjacent teeth will allow “creeping” of the interproximal gingival tissue and filling the empty space. In case of abnormal tooth shape or missing teeth, prosthetic restoration can help creating adequate contacts which will possibly lead to papilla formation, given that the biological width and the supracrestal attachment of collagen fibers is respected [10, 11]. Surgical approaches include different methods for papilla reconstruction. Techniques of pedicle flaps [12], semi-lunar coronally reposi- tioned flaps [13] and regenerative procedures with connective tissue and bone grafts, often combined with a micro- surgical approach are used [14, 15, 16].

However, a predictable technique for the reconstruction of lost papillae remains elusive. One of the major reasons for this is the limited blood supply of the papilla, which is a problem for any form of grafting. Tunneling surgical approach eliminates the need for releasing incisions, which hinder the blood supply in this delicate area and improve vascularity and hence – better post-surgical outcomes are reported. However, all surgical techniques are technically demanding, with unpredictable regeneration of the papillae and cannot offer successful long-term outcomes.

In order to offer a treatment method, which is less invasive and offer more predictable results, tissue volumizing techniques with bioactive molecules were proposed. Currently for this purpose is used mainly hyaluronic acid. Hyaluronic acid (HA) (or hyaluronan) is a natural component of the extracellular matrix with various structural and physiological functions, which maintain the homeostatic integrity of the tissues. Its biological functions include cellular and intercellular interactions, modulation of inflammation and periodontal wound healing, stimulation of angiogenesis and collagen synthesis. It is non-immunogenic, biocompatible and biodegradable – properties, which makes it ideal for biomedical usage. Modifications of HA molecules by crosslinking improves these mechanical properties by creating a gel with firmer structure and rigidity, suitable for clinical applications, as well as extended clinical efficacy by 6-12 months [17].

Recently some data reporting results after HA injections was published. A study by Becker et al evaluated the results of 14 treated sites after HA gel injection 2-3 mm under the tip of the papilla up to three times at three weeks intervals. Their results presented evidence, that small papillary deficiency between implants and teeth can be enhanced by HA and the improvement was maintained for up to 25 months [18]. Other clinical trials also evaluated the ability of injectable HA gel to fill the black triangles and most of them reported sufficient papilla fill, with an average follow-up of 6 months [19, 20, 21]. However, studies with long-term results are still not available.

Therefore, the aim of our study was to evaluate the effect of HA gel injections in deficient papillae and monitor the results for a 6-months period.

Material and methods: 19 patients (aged 23-72 years old) with 57 deficient papillae were included in the study. Inclusion criteria were:

- Non-smokers;
- No uncontrolled systemic diseases or administration of drugs, which could affect gingival condition (antihypertensive medications, etc);
- Maxillary anterior teeth;
- Teeth without caries lesions, fillings, veneers or prosthetic crowns;
- Plaque index less than 15%;
- Class I and class II papillary deficiency;
- No diastema, caries, proximal restorations, fixed prosthesion or orthodontic appliances;
- The distance between the contact point and alveolar bone crest should be 5 mm or less.

Subjects with medical conditions, which could affect periodontal healing were excluded, as well as patients with any drug intake that could affect the gingiva; history of allergy or periodontal surgeries in the past 6 months.

All included patients signed written consent with the full agreement of participation in this study.

For the purpose of this study, we used hyaluronic acid gel, composed of a mixture of cross-linked (1,6%) and natural (0,2%), marketed as HyaDent BG (BioScience, Germany).
There is no specific and strict method of injection of HA in the gingiva. However, Gottfert and Striegel [22] proposed a 3-step technique as follows:

Step 1 - identifying the treating the underlying cause, if possible, because it would affect the overall treatment and long-term outcome and stability of the results;

Step 2 - for class I and class II papilla deficiency injection of HA into the papilla, 3-4 mm apical to the tip. Quantity is individual and should be tailored according to each case, depending on the volume of lost tissue. For class III papilla deficiency multiple drops of HA should be injected 3-4 mm below the tip of the papilla

Step 3 - application of a few droplets of HA topically to the injected area to enhance the healing environment topically.

HA gel can be applied not only in the papilla but also in the tissues above the defect, into the muco-alveolar junction and in the attached gingiva:

Step 1 - injection of HA into the muco-alveolar junction above the deficient papilla. The injection volume is tailored to the situation;

Step 2 - injection into the attached gingiva above the deficient papilla;

Step 3 - injection 2 mm below the highest point of the papilla to stabilize the papilla itself.

We could not find any data, supporting the advantages of this additional HA gel injection, when the main goal is papilla augmentation. That is why we used the technique of gel injection directly into the base of the papilla after local anaesthesia using infiltration technique was applied before the injection. The gel was injected 2-3 mm apical to the papilla tip, with a 23 gauge needle, directed coronally. The volume of the injected gel was tailored individually, till whitening of the adjacent tissue was noticed. Then few droplets of HyaDent BG were topically applied and massages onto the treated area. The patient was instructed not to brush for 24 hours, then start the routine oral hygiene measures, avoiding using dental floss at the treatment sites. The injection was repeated after 20 days.

The patients were monitored 1, 3 and 6 months post-operatively. Measurements of papilla height were done on the clinical photographs, which were taken under the same conditions at each visit with the same digital camera. The photographs were taken under the same lighting conditions, using the same lens and the same focal length. The patients were sitting in an upright position, looking straight ahead. Care was taken to ensure that the same shooting positions were reproduced at each time visit. Each site was individually calculated. We measured in millimeters the distance between the contact point (complete fill of interproximal space) to the most coronal level of the visible gingival margin – this measurement is actually the vertical size of the black triangle formed. The papilla change on months 1, 3 and 6 was compared to baseline. Clinical photographs of papillae before and after treatment are shown on fig. 2, 3, 4 and 5.

Fig. 2 a, b, c. Clinical view of the patient with black triangles between central and lateral incisors, before injection.

Fig. 3. 1 month following 1st injection

Fig. 4. 3 months follow-up

Fig. 5. 6 months follow-up.

Statistical Analysis

The statistical analysis was performed with the Statistical Package for the Social Sciences (SPSS), Version 26 (2018). The gain in papilla height was expressed as means ±SD and as percent change from baseline to the sixth month after treatment with four-time points: baseline, one month, three months and six months after treatment. The Shapiro-Wilk’s test showed that the assumption of normality was observed at all time points (p > 0.05 for baseline, 1 month, 3 months and 6 months). In order to track the change in papilla height (respectively the reduction of the black triangle) we performed paired-samples t-tests to compare the mean values and percent gain at the different points of time. Fisher’s exact test was employed in the statistical comparison of proportions. The results were interpreted as statistically significant at p < 0.05.
RESULTS
The t-test paired comparisons showed a significant papilla gain between all time intervals (Table 1). At baseline, the papilla height had a mean of 3.82 mm, which was reduced to 1.68 mm in the first month after the treatment, p < 0.001. A significant reduction in papilla height was also observed in the third month (1.13 mm), p < 0.001; and at the sixth month (0.95 mm), p = 0.028. In total, a significant reduction in papilla height of 1.92 mm was observed between baseline and the sixth month following treatment, p < 0.001.

Table 1. Reduction of black triangles and change in papilla height in mm between baseline and six months after the injection of HA gel

<table>
<thead>
<tr>
<th>Time of measurement</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>95% Confidence Interval</th>
<th>Paired-samples t-test p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower Bound</td>
<td>Higher Bound</td>
</tr>
<tr>
<td>Baseline a</td>
<td>56</td>
<td>3.82</td>
<td>0.97</td>
<td>3.57</td>
<td>4.1</td>
</tr>
<tr>
<td>One month b</td>
<td>56</td>
<td>1.68</td>
<td>1.11</td>
<td>1.37</td>
<td>1.98</td>
</tr>
<tr>
<td>Three months c</td>
<td>55</td>
<td>1.13</td>
<td>0.88</td>
<td>0.89</td>
<td>1.37</td>
</tr>
<tr>
<td>Six months d</td>
<td>56</td>
<td>0.95</td>
<td>0.74</td>
<td>0.76</td>
<td>1.16</td>
</tr>
</tbody>
</table>

*a vs. b: < 0.001**
*b vs. c: < 0.001**
*c vs. d: 0.028*
*a vs. c: < 0.001**
*a vs. d: < 0.001**

*-Significant at p <0.05;**-Significant at p < 0.01

The statistically significant continuous trend of reduction of the black triangles and in the increase of papillary height (papilla gain) from baseline to the sixth month after the treatment is illustrated in figure 6.

Fig. 6. Reduction of the black triangle area and the papilla gain from baseline to the sixth month after treatment

**/*- Based on paired-samples t-test

The percent of papilla gain between baseline and at one, three and six months after treatment was also significant (Fig. 7). We observed 59% papilla gain at month one (p < 0.001), which increased significantly to 72% at month three (p< 0.001) and further increased to 77% at month six (p = 0.027).
Fig. 7. Percent change in papilla gain from baseline to the sixth month after treatment

**/*- Based on paired samples t-test

Tracing the dynamics in the percent papilla gain (fig. 8) revealed an improvement rate, ranging between 25% and 100% at month one following treatment. Nine patients had 25% improvement and 10 patients reached 100% improvement. In the second follow-up, three months later, the range remained the same (25% to 100%), but the number of patients with 25% papilla gain decreased from 9 to 1, and those with 100% gain increased from 10 to 15. Six months after the treatment, the improvement rate shifted upwards, with the lowest gain at 50%. The distribution of the patients across improvement rates at the sixth month, following treatment, included: 5 patients with 50% papilla gain; 8 patients with 60% gain; 9 with 67% gain; 13 with 75% gain; and 17 with 100% gain.

Fig. 8. Dynamics in percent papilla gain from baseline to the sixth month after treatment.
Expressed in percentages, the distribution of patients according to the rate of papilla gain at the sixth month following treatment was the following: 31% of the patients had 100% gain; 7% had 80% gain; 23% had 75% gain, 16% had 67% gain; 14% had 60% gain, and 9% had 50% gain. The patients with improvement rate between 75% and 100% constituted a significantly higher percentage (61%) of the study group vs. 39% with an improvement rate between 50% and 70%. None of the patients at the sixth month visit following treatment had a gain of papilla below 50% (fig. 9).

**Fig. 9.** Distribution of the patients across percent papilla gain at the sixth month after treatment

Our results provide evidence in support of the effectiveness of injected hyaluronic acid gel in the treatment of deficient papillae, which could be an esthetic (and might be – a phonetic) problem for patients. We observed a significant and continuous trend of reduction of the black triangles area and a significant gain in the papillary height from the baseline to the sixth month following HA augmentation. The overall reduction of the black triangles at the sixth month was estimated at 77%, with the majority of the patients (61%) reaching improvement between 75% and 100%. None of the patients had a papillary gain below 50%.

**Fig. 10 a, b.** Patient with advanced periodontitis and papilla loss.

However, in cases with advanced Class III papilla loss injection of hyaluronic acid did not lead to clinical visible improvement, as shown in the case in fig. 10 a and b. The patient had previous periodontal surgery in the anterior maxillary area. The distance between contact-point and the alveolar crest is far beyond 5 mm, as seen in fig. 11. We were able to achieve some positive change in the volume of the papillae between right lateral incisor and canine, but no visible effect was noticed in terms of papilla augmentation between central and lateral incisors.
DISCUSSION.

The present clinical study was performed to evaluate the effect of HA gel injection in deficient papillae as a minimally invasive method of treatment, which might reduce the visible black triangles in the anterior esthetic zone and enhance the “pink” esthetics. Results from this study are encouraging. 1 month after the HA gel injection, a papilla gain and a reduction of the visible black triangles were obvious in 59% of the treated sites. In the follow-up period, up to the 6th month, constant improvement was observed, in terms of papillary gain and augmentation, as well as a volumizing effect. In addition, the procedure was very well tolerated by the patients and reported to be a not painful procedure. Most of them were very satisfied with the achieved esthetic results. Even in cases where a complete fill of the black triangles was not observed, patients were satisfied (given that they understood the anatomical base of the initial situation). However, the achieved augmentation in those cases had some additional motivating effect on their willingness to take further care of their periodontal health.

Our results are generally consistent with the previously conducted studies by Awartani et al. [19] Mansouri et al. [21] and Abdelraouf et al. [23], although they reported only the mean percentages of reduction of the black triangle surface area.

In our study, the high percentage of papilla fill is attributed mainly to the comparatively small size of papilla loss, with only class I and class II deficient papillae being included. Since papillae can be lost due to many etiologic factors, they must be cleared and eliminated, if possible, before HA injection is applied. Obviously, HA gel is not able to refill and reconstruct big deficiencies, as demonstrated above. Such clinical situations should be taken into consideration when dentists plan minimally invasive augmentation. A realistic prognosis should be presented to the patients for the expected treatment outcomes and the possibility of re-injection of HA in the deficient papillae should be explained.

Papilla gain probably is not a simple result of tissue filling and volumizing effect. HA has multiple pleiotropic biological effects in the tissues. Stimulation of fibroblast activity and collagen synthesis, along with angiogenesis, are the physiologic base for papilla regeneration. Further investigations are necessary to identify which type of modified HA, what quantity and time intervals for re-injection are optimal for the long-term stability of achieved results. Injections in receded peri-implant tissues with long-term follow up is another challenging perspective.

CONCLUSION

Minimally invasive reconstruction of lost papillae with hyaluronic acid is an easy, affordable and predictable technique, which satisfy the esthetic demands of both patients and dentists for a period of at least 6 months. Minimally invasive management of deficient papillae with hyaluronic acid responds to the growing public demands for esthetics and harmonious smile of patients.
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