

# Histologic evaluation of 5% Hyaluronic acid injection in oral tissue: A pilot study

## Abstract

**Background:** Hyaluronic acid is a naturally derived, nonimmunogenic, nonadhesive glycosaminoglycan that plays a prominent role in various wound healing processes, as it is naturally angiogenic when degraded to small fragments.

**Material and methodology:** A case report was conducted on one male patient with operculum present bilaterally. Left side operculum was excised at baseline. The same day of excision, 5 % hyaluronic acid was injected on the right-side operculum which was excised on 15<sup>th</sup> day for post HA injection evaluation.

**Results:** Epithelium showed stratified squamous para-keratinization with focal epithelial hyperplasia entrapped with connective tissue. The connective tissue was moderately fibrous with focal areas of cellularity showing plump fibroblasts and fibrocytes.

**Conclusion:** Changes seen from pre to post HA injection are indicative of initial step towards increasing the size of a tissue clinically.

**Keywords:** hyaluronic acid, histology, epithelium, connective tissue

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## Introduction

Hyaluronic acid (HA) is a high molecular weight biopolysaccharide, discovered in 1934, by Karl Meyer and his assistant, John Palmer in the vitreous of bovine eyes. Hyaluronic acid is a naturally occurring biopolymer, which has important biological functions in bacteria and higher animals including humans. It is found in most connective tissues and is particularly concentrated in synovial fluid, the vitreous fluid of the eye, umbilical cords and chicken combs. It is naturally synthesized by a class of integral membrane proteins called hyaluronan synthases and degraded by a family of enzymes called hyaluronidases.<sup>1</sup>

Hyaluronic acid (HA) plays a vital role in the synthesis of extracellular matrix molecules and epidermal cell interaction with the surrounding environment. It modulates cellular immunity by preventing infections and impeding allergic phenomena. One of its most important properties is that it can attach and hold large amounts of moisture; approximately 6L of water in just 1g. Hyaluronic acid is said to be an ultimate solution for moisture retention of the skin. Youthful skin is hydrated because it contains large amounts of HA in the dermis. However, as we age, the amount of HA in the skin decreases and by the time we become adults, this amount decreases to five percent of baseline.<sup>2</sup>

The challenging characteristic of conventional HAs in the use of topically applied anti-aging preparations has been that its molecules are 3,000nm in diameter, whereas the intercellular space is only 15 to 50nm and just 6 to 10nm at the hyaline membrane. This makes it impossible for conventionally produced HA to penetrate into deep layers of the dermis.<sup>3</sup> There are several uses of hyaluronic acid in medical and dental field. In medical field it is used in facial rejuvenation,<sup>4</sup> ophthalmology,<sup>5</sup> osteoarthritis,<sup>6</sup> bone formation,<sup>7</sup> TMJ disorders,<sup>8</sup> otolaryngology,<sup>9</sup> cardiovascular disease,<sup>10</sup> atrophic vaginitis,<sup>11</sup> diabetic foot ulcer,<sup>12</sup> peripheral nerve regeneration.<sup>13</sup> In

dental field it is used in treatment of gingivitis,<sup>14</sup> periodontitis,<sup>15</sup> gingival recession,<sup>16</sup> in intrabony defects,<sup>17</sup> oral lichen planus,<sup>18</sup> recurrent aphthous ulceration,<sup>19</sup> actinic cheilitis,<sup>20</sup> implants,<sup>21</sup> sinus lift,<sup>22</sup> in healing of extracted tooth sockets,<sup>23</sup> through tissue engineering in gingival augmentation<sup>24</sup> and dental pulp regeneration.<sup>25</sup>

The use of hyaluronic acid in cosmetology for skin treatment is well known. However, the mechanism of action of HA after dermal injection is minimally explained. Recently, the use of HA injection for IDP deficiency treatment is in demand and there are few studies<sup>26-30</sup> on its clinical effect. The skin anti-ageing treatment with hyaluronic acid is used effectively. However, the histologic evaluation of such ha injection in skin is minimally reported. Based on the dermal use of hyaluronic acid, successful clinical trial on 5% HA<sup>31</sup> has been reported and prompted us to conduct the histologic evaluation in oral tissues to comprehend the mechanism of its action.

However, the histologic study of HA injection to understand its mechanism of action during IDP deficiency treatment has not been done so far. Hence, the present case-report was the first attempt to histologically evaluate the gingival tissue following 5% HA injection.

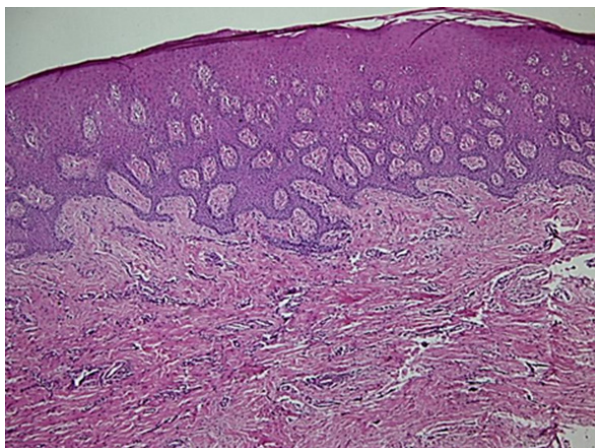
## Methodology

In the current study attempt has been made to study the histological changes in oral tissue (operculum) 15 days after hyaluronic acid (HA) injection. A case report was conducted on one male patient with operculum present bilaterally. Left side operculum was excised at baseline. The same day of excision, 5 % hyaluronic acid was injected on the right side operculum which was excised on 15<sup>th</sup> day for post HA injection evaluation. Immediately after excision, tissue was transferred to 10% formalin and sent to oral pathology department for histologic examination. Staining was done with H&E stain and light microscope was used to evaluate the microscopic epithelium and connective tissue changes.

## Results

### Histo-pathological findings before Hyaluronic acid injection

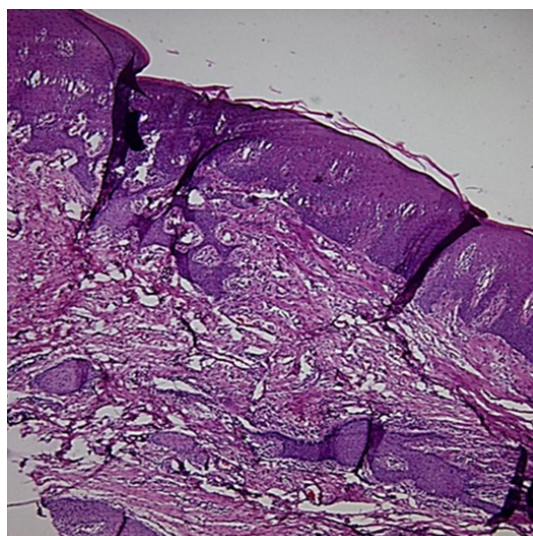
Epithelium showed Stratified squamous para-keratinized with entrapped connective tissue. The connective tissue showed fibrous with few fibroblasts and fibrocytes interrupted with blood vessels. It also showed few lymphocytes, mast cells and neutrophils. Salivary acini, adipose tissues, nerve bundles and extravasated RBCs were also seen (Figure 1).



**Figure 1** Histological picture at baseline.

### Histo-pathological findings after Hyaluronic acid injection

Epithelium showed stratified squamous para-keratinization with focal epithelial hyperplasia entrapped with connective tissue. The connective tissue was moderately fibrous with focal areas of cellularity showing plump fibroblasts and fibrocytes with interspersed blood vessels. Inflammatory cells composed of lymphocytes, plasma cells, few neutrophils were seen. Areas of extravagated RBCs were also seen (Figure 2).



**Figure 2** Histological picture after 15 days of post hyaluronic injection.

## Discussion

Hyaluronic acid is a naturally derived, non-immunogenic, non-adhesive glycosaminoglycan that plays a prominent role in various wound healing processes, as it is naturally angiogenic when degraded to small fragments. Hyaluronic acid promotes early inflammation which is critical for initiating wound healing, but then moderates later stages of the process, allowing matrix stabilization and reduction of long-term inflammation.<sup>32</sup> Functions of HA include the following: hydration, lubrication of joints, a space filling capacity, and the framework through which cells migrate.<sup>33</sup> The synthesis of HA increases during tissue injury and wound healing<sup>34,35</sup> and HA regulates several aspects of tissue repair, including activation of inflammatory cells to enhance immune response<sup>36</sup> and the response to injury of fibroblasts<sup>37</sup> and epithelial cells.<sup>38</sup> HA also provides the framework for blood vessel formation<sup>39</sup> and fibroblast migration,<sup>40</sup> that may be involved in tumor progression. The correlation of HA levels on the cell surface of cancer cells with the aggressiveness of tumors has also been reported.<sup>41</sup>

The histologic report of the current study suggests that the overall changes seen from pre to post HA injection were epithelial hyperplasia and moderately fibrous connective tissue with focal areas of fibroblast cellularity which are indicative of initial step towards increasing the size of a tissue clinically. The histologic HA effects were evaluated at 15 days in a single patient so that both pre and post HA injection tissue response can be appreciated from the same patient. The case report represents the first of the histologic effect of HA after gingival injection which serves as the basis for understanding the clinical voluminising effect of hyaluronic acid. The shortcoming of this pilot study was lack of patient's willingness to participate readily. Further histologic evaluation can be done to enhance the current study report using the similar methodological protocols in larger sample size participants.

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None.

## Ethical consent

None.

## Conflicts of interest

The author declares there are no conflicts of interest.

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