THE EFFECT OF HYALURONIC ACID ON GINGIVAL INFLAMMATION AND GINGIVAL BLEADING DURING GBR

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Abstract. *The management of periodontal defects has been an ongoing challenge in clinical periodontics. This is mainly a result of the fact that tissues which comprise the periodontium, the periodontal ligament, and the cementum and alveolar bone, represent three unique tissues in their own right. Thus, reconstruction of the periodontium is not just a simple matter of regenerating one tissue but involves at least three quite diverse and unique tissues. Resective surgical therapy, with or without osseous recontouring, was considered the norm during the 1950s and the 1960s, in the belief that attainment of shallow pocket depths was a worthwhile goal. More recently, attention has been focused more on regenerative and reconstructive therapies, rather than on respective therapies. Among the many mediators used in periodontal regeneration is hyaluronic acid. In the field of dentistry, hyaluronic acid has shown anti-inflammatory and anti-bacterial effects in the treatment of periodontal diseases. The article reviews recent evidence of the effects of hyaluronic acid on periodontal deffects.*

Keywords: *hyaluronic acid, bone regeneration, periodontal regeneration and reconstructive therapies, gingival inflammation and bleading.*

1. INTRODUCTION

Hyaluronic acid has been identified in all periodontal tissues in varying amounts and is more pronounced in non-mineralized tissues, such as the gingival and periodontal ligaments, compared with mineralized tissues such as cement and alveolar bone. In addition, due to the high levels of hyaluronic acid in the circulating blood serum, it is constantly present in the gingival blood flow fluid (GCF) which is a factor in serum overload[1] . Natural hyaluronic acid is an extremely hydrophilic polymer that exists, as viscous does not in itself have the structural features needed for use as a surgical product. Hyaluronic acid ester synthesized by esterification of a carboxyl group with benzyl alcohol is less soluble in water and is therefore more stable. Due to its unique molecular structure, hyaluronic acid can accumulate at different molecular weights such as lyophilized or esterified in different structural configurations such as membranes. The rate of biodegradation of these materials can be manipulated by changing their degree of lyophilization or esterification. Thus, hyaluronic acid may be useful as a reproductive material in regenerative surgical procedures[2] . Hyaluronic acid is an anionic, glycosaminoglycanic acid widely distributed throughout connective, epithelial, and nerve tissues. It is unique among glycosaminoglycans in that it is non-sulfated and forms in the plasma membrane instead of in the Golgi apparatus. The human synovial hyaluronic acid averages about 7 million daltons per molecule, or about twenty thousand disaccharide monomers, while other sources mention 3-4 million daltons. One of the major components of the extracellular matrix, hyaluronic acid, contributes significantly to cell proliferation and migration, and may also be involved in the progression of some malignancies. The average 70 kg person has approximately 15 grams of hyaluronic acid in the body, of which one third is degraded and synthesized every day[3] . Hyaluronic acid is also a component of group A streptococcal extracellular capsule, and is believed to play an important role in virulence.Hyaluronic acid is one of the most well-known hygroscopic molecules known in nature.

When HA is incorporated in aqueous solution, hydrogen bonding occurs between adjacent carboxyl and N-acetyl groups; this feature allows hyaluronic acid to maintain conformational stiffness and retain water. One gram of hyaluronic acid can bind up to 6 L of water. As a physical material, it has functions in spatial filling, lubrication, shock absorption, and protein exclusion[4]. The viscoelastic properties of the material can slow down the penetration of viruses and bacteria, a feature of particular interest in the treatment of periodontal disease. Hyaluronic acid as a viscoelastic substance helps in periodontal regenerative procedures by maintaining spaces and protecting surfaces[4] . By recognizing its hygroscopic and viscoelastic nature, hyaluronic acid can affect cell function by modifying surrounding cellular and extracellular micro and macro media. The hyaluronic acid has many structural and physiological functions within tissues, including extracellular and cellular interactions, the interaction between the growth factor and the regulation of osmotic pressure, and tissue lubrication, which helps maintain the structural and homeostatic integrity of tissues[5] .

Considering the various beneficial effects of hyaluronic acid, we focused our interest on the review of the effects of hyaluronic acid on gingival bleading and gingival inflammation in guided tissue regeneration.

2. Materials and Methods

30 patients were followed in the study. Patients were selected according to the following criteria:

- aged between 20 - 45 years
- without anamnestic data for general disease
- non-smokers
- -finding of both contralateral similar type of periodontal destruction both in volume and type
- All will have a clinical examination before the intervention and then will be measured:
- depth of the periodontal pocket,
- loss of attachment,
- recession,
- gingival bleeding and gingival inflammation (measurements will be made on a Silnes Loë gradient)

Prior to the intervention, all patients were given advice on proper oral hygiene and it will be checked whether they implemented. The modified Widmann method was made for all patients, with BioOss beef bone (control group) applied to each patient on one side and BioOss with 16% BDDE hyaluronic acid from Stylage, Vivacy, applied on the other side. Paris in a ratio of 2: 1, enough until a thick, sticky bone ratio is obtained - (examined group). In all patients, the results were monitored by CBCT-conbim (computed tomography in which X-rays are divergent to form a cone beam) and measurements will be made before and after 12 (twelve) months.

3. Results

Gingival inflammation in the studied group in different control periods

	x	**sd**	**t**	**p**
begining	**2,11**	**0,55**		
After 3 months	**0,78**	**0,18**	**12,41**	**≤0,05**
After 6 months	**0,44**	**0,25**	**14,76**	**≤0,05**

After 8 months	**0,33**	**0,45**	**14,09**	**≤0,05**

Gingival inflammation in the control group in different control periods

	x	**sd**	**t**	**p**
begining	**2,33**	**0,68**		
After 3 months	**1,56**	**0,48**	**5,75**	**≤0,05**
After 6 months	**1**	**0,23**	**11,135**	**≤0,05**
After 8 months	**0,78**	**0,63**	**10,81**	**≤0,05**

Difference in gingival inflammation index values between baseline and control periods for study and control groups

	Study group	**Control group**	**p**
After 3months	**0,77**	**1,33**	**≤0,0001**
After 6 months	**1,33**	**1,67**	**0,027**
After 8 months	**1,55**	**1,78**	**1.337**

The differences between the initial values and the different time periods for the index of gingival inflammation were; for the control period of 3 months o.77 for the examined and 1.33 for the control, for the period of 6 months 1.33 for the examined and 1.67 for the control while for the period of 8 months 1.55 for the examined and 1.78 for the control. The difference is significant for the third month p ≤0.05.

Gingival bleeding in the studied group in different control periods

	x	**sd**	**t**	**p**
begining	**2,67**	**0,23**		
After 3 months	**1,67**	**0,23**	**11,81**	**≤0,05**
After 6 months	**0,56**	**0,25**	**24,26**	**≤0,05**

After 8 months	**0,67**	**0,45**	**19,29**	**≤0,0001**

Gingival bleeding in the control group at different control periods

	x	**sd**	**t**	**p**
begining	**2,56**	**0,25**		
After 3 months	**1,89**	**0,11**	**8,928**	**≤0,05**
After 6 months	**1,44**	**0,25**	**12,449**	**≤0,05**
After 8 months	**1,11**	**0,33**	**15,092**	**≤0,05**

Difference in gingival bleeding index values between baseline and control periods for study and control groups

	Study group	**Control group**	**p**
After 3 months	**1**	**0,67**	**≤0,026**
After 6 months	**2,11**	**1,12**	**≤0,0001**
After 8 months	**2**	**1,45**	**≤0,00001**

The differences between the initial values and the different time periods for the index of gingival bleeding is for the control period of 3 months 1 for the subject and 0.67 for the control, for the period of 6 months 2.11 for the subject and 1.12 for the control while for the period of 8 months 2 for the tested one and 1.45 for the control one. The difference is significant for the sixth and eighth months $p \leq 0.05$.

4. DISCUSSION

Today HA is widely used in many branches of medicine with interesting potential applications in dentistry for the treatment of acute and chronic inflammatory disease. Data obtained from the present review of 20 clinical studies demonstrates that, due to its positive action on tissue repair and wound healing, topical administration of HA could play a role not only in postoperative dental surgery, but also in the treatment of patients affected by gingivitis and periodontitis, with a significant improvement in their quality of life. Further, laboratory-based research and large-scale randomized controlled clinical trials on a larger scale are advisable to confirm these promising results. From the perspective of current research, hyaluronic acidbased bone regenerative scaffolds are more biocompatible and

bioactive with biomimetic strategies. As a matrix component, hyaluronic acid, especially sulfated HA, may trigger cell behavior modulation via several signaling pathways, leading to faster and more desirable bone formation. Scaffolds and carriers based on HA are shaped into either rigid forms or colloids. As a rigid scaffold material, when incorporated with other materials, HA may alter the scaffold morphology and improve mineralization, making it more desirable and more functional for bone regeneration. Moreover, hyaluronic acid is chemically versatile, with its properties changed via simple chemical modification and crosslinking. The viscidity, rheological properties, pH, and charge properties of hyaluronic acid can be modulated into states suitable for gelation or delivery. This leads us to the carrier hyaluronic acid. Either by mixing, or by chemically or electrostatically encapsulating a diverse range of growth factors, drugs, mineralized components, or cells in HA-based carriers, bone formation can be markedly enhanced and accelerated. New bone formation could more closely resemble that of the original tissue. Some strategies can also perform superbly in Osseo integration for implantation. HA-based hydrogels and micro particles can covalently bind to metal implant surfaces and release bioactive components, resulting in better osteogenesis and Osseo integration. However, the specific mechanisms behind the effects of HA on osteogenesis still require proper investigation controlled delivery as well as biomimetic scaffold and carrier designs, not just HA-based forms. Bone regeneration in periodontal bony defects.More recently, cross-linked HA products were used as gel barriers to cover the osseous defects around the implants and implant recipient sites and thereby promoting GBR [6] . Claar performed a lateral coverage of the augmentation followed by use of cross-linked HA in gel form, which was developed especially for GBR[7]. The principles of GBR applications[8, 9] are as follows: - Cell exclusion: Creating a barrier to prevent forming fibrous connective tissue by epithelial cells. - Tenting: New wound space beneath the membrane must be regenerated solely around soft tissues so that high quality of new tissue can be gained. - Scaffolding: At first, a fibrin clot is seen in this space which is a scaffold for progenitor cells. Adjacent hard tissues serve as storage for stem cells. - Stabilization: To gain successful healing, the defective area must be protected from environmental effects such as flap movement, bacterial invasion, exposure of region, etc. by fixing the membrane into position.

The findings of our investigation clinical study indicate that the use of HA + GTR as a regenerative material was found to be effective in improving the clinical parameters compared to GTR alone, also we prove that first 3 months we have more control on gingival bleading and gingival inflamation , who representiv key of succesful periodontal regeneration. Engström *et al*. investigated the anti-inflammatory effect and the effect on bone regeneration of Hyaluronan in surgical and non-surgical groups. No statistical difference was found on radiographs in the non-surgical group, whereas the decrease in bone height was found for both groups after scaling. Probing depth (PD) reduced after the surgical treatment as well as after scaling and root planning (SRP). Hyaluronan in contact with bone and soft tissues had no influence on the immune system.[10]

In our present study, the differences between the initial values and the different time periods for the index of gingival inflammation were; for the control period of 3 months o.77 for the examined and 1.33 for the control, for the period of 6 months 1.33 for the examined and 1.67 for the control while for the period of 8 months 1.55 for the examined and 1.78 for the control. The difference is significant for the third month $p \leq 0.05$. Hyaluronan gel is effective in controlling inflammation and gingival bleeding. Studies have documented reduction in the depth of gingival pockets along with a significant reduction in epithelial and lymphocyte cell proliferation with the use of HA gel.[11]

0.2% Hyaluronan containing gel has a beneficial effect in the treatment of plaque induced gingivitis. All studied sites had a significant decrease in peroxidase and lysozyme activities after 7, 14, and 21 days.[12] Hyaluronan gel is also effective in controlling inflammation and gingival bleeding. Pilloni *et al*., in their randomized controlled clinical pilot study, evaluated the efficacy of an esterified form of HA gel on periodontal clinical parameters. The periodontal clinical parameters were plaque index (PI), BOP, PPD, gingival index (GI), and probing attachment level. In the end of the study, they concluded that an esterified gel form of HA has shown an effect in reducing the gingival inflammation when used as an adjunct to mechanical home plaque control and that it could be successfully used to improve the periodontal clinical indexes.[13]

Also in our study, we presentiv the differences between the initial values and the different time periods for the index of gingival bleeding is for the control period of 3 months 1 for the subject and 0.67 for the control, for the period of 6 months 2.11 for the subject and 1.12 for the control while for the period of 8 months 2 for the tested one and 1.45 for the control one. The difference is significant for the sixth and eighth months $p \leq 0.05$. Therefore in our present study, radiographic monitoring of alveolar bone changes was carried out as end point variable. The most reliable outcome variable for assessing periodontal regeneration is human histology. Due to ethical considerations and patient management limitations, no histological evidence was obtained to establish the proof of periodontal regeneration. The importance of wound stability for bone and periodontal regeneration has been reported, thanks on stability of gingival inflamation and bleading controlled by hyaluronic acid. Based on the histological evidence from human material, it may be assumed that the clinical improvements following esterified HA treatment may represent at least to some extent, a real periodontal regeneration characterized by the increase of osteoblastic activity by stimulating differentiation and migration of mesenchymal cells.[14] Moreover, the physiochemical properties of HA help keep the growth factors responsible for tissue repair in situ.[15]

5. CONCLUSION

The results of this research are expected to contribute to the knowledge of the impact of hyaluronic acid on periodontal regeneration and its application in the daily life of periodontology.

The obtained research results together with the data from the literature give us relevant knowledge for optimal scientifically supported planning and realization for successful periodontal treatment. The scientific contribution of this research is the optimism that the scientific findings from this research will arouse interest and need for new research on this issue. From the analysis of the results and within the limitations of the present study, it can be concluded that regenerative approach using hyaloss in combination with GTR for the treatment of human infrabony defects resulted in a significant added benefit of anti inflamatory proces who prese nt no gingival bleading and inflamation in first 3 months , they build the succes of periodontal regeneration , in terms of CAL gains, PPD reductions and radiographic defect fill, as well as LBG, compared to the GTR alone.

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