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HYALURONIC ACID: A PROMISING MEDIATOR FOR PERIODONTAL REGENERATION

ХИЈАЛУРОНСКА КИСЕЛИНА-ВЕТУВАЧКИ МЕДИЈАТОР ЗА ПАРОДОНТАЛНА РЕГЕНЕРАЦИЈА

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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 ishyaluronic 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 tissue. **Keywords:** hyaluronic acid, bone regeneration, periodontal regeneration and reconstructive therapies.

Апстракт

Справувањето со пародонталните дефекти претставува постојан предизвик во клиничката пародонтологија. Предизвикот е поголем со согледување на фактот дека ткивата што го сочинуваат пародонтот, пародонталните лигаменти, цементот и алвеоларната коска претставуваат посебни уникатни ткива со свои специфики метаболна динамика и регенеративни потенцијали. Така, реконструкцијата на пародонтот не е само едноставна работа за регенерирање на едно ткиво, туку вклучува најмалку три прилично разновидни и уникатни ткива. Додека ресективната хируршка терапија се сметаше за норма во текот на 1950-тите и во 1960-тите години, во поново време вниманието е насочено повеќе кон регенеративните и реконструктивните терапии. Покрај многубројните материјали кои се користат, суште не постојат доволно податоци за да се даде предност на одредени супстанци кои ќе доведат до подобра регенерација на ткивата
Во последниве години хијалуронската киселина се повеќе се користи како медијатор, тоа е материјал кој се аплицира во пародонталниот дефект со цел да предизвика регенерација на коските. Во областа на стоматологијата, хијалуронска киселина апокажа анти-инфламаторно и антибактериско дејство во третманот на пародонталните болести. Иако подолго време се користи во различни области на стоматологијата, сеуште докрај не е проследено влијанието на хијалуронската киселини врз ткивата на пародонтот. Токму затоа и одлучивме да направиме согледување на досегашните резултати во однос на примената и ефектите од хијалуронската киселина во пародонтологијата. **Клучни зборови:** хијалуронскакиселина, коскена регенерација, пародонтална регенерација и реконструктивни терапии.

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 periodontal tissue

Material and Methods

The purpose of this paper was to systematize available data on effects of hyaluronic acid on periodontal tissues. The survey was conducted through Pubmed-medline database using key words periodontal tissue, periodontal regeneration, and hyaluronic acid.

Results and discussion

In the early inflammatory phase of wound healing, HA is abundant as if it were a damaged tissue, probably a reflection of the increased synthesis. The HA acts as a promoter of early inflammation, which is crucial in the whole process of skin wound healing. In the model as a bag for air odors of carrageenan / IL-1-induced inflammation, HA has been observed to improve cell infiltration. (6,7) showed a dose-enhancing dose of proinflammatory cytokines TNF-α and IL-8 production from human stem fibroblasts at concentrations of HA from 10 μg / ml to 1 mg / mL via a CD44-mediated mechanism. Endothelial cells, in response to inflammatory cytokines such as TNF-α and bacterial lipopolysaccharide, also synthesize hyaluron, which has been shown to facilitate primary adhesion to cytokine-activated lymphocytes, which express hyaluronic acid binding variants, and static flow[8]. HA has contradictory dual functions in the inflammatory process. Not only it can promote inflammation, as noted above, but it can also calm the inflammatory response, which can help stabilize the tissue matrix during granulation, as described in the next section. An integral part of the formation of granulation tissue, in order to proceed with normal tissue repair inflammation needs to be modelled. Initially, granulation tissue is highly inflammatory with high rate of tissue turnover mediated by enzymes that degrade matrices and reactive oxygen metabolites that are products of inflammatory cells[6]. Stabilization of the granulation tissue matrix can be achieved with moderate inflammation. HA functions as an important moderator in this process of moderation, in contrast to its role in inflammatory stimulation, as described above. HA can protect against free radical damage to cells[9]. This can be attributed to its property of free radical scavenging, a physicochemical characteristic shared by large polymer polymers. In the rat model of free radical scavenging, HA was shown to reduce granulation tissue damage[10]. In addition to the role of a free-radical task, HA can function in a negative feedback loop from inflammatory activation through specific biological interactions with the biological constituents of inflammation[6]. TNF-α, an important cytokine generated in inflammation, stimulates the expression of TSG-6 (TNF-stimulated gene 6) in fibroblasts and inflammatory cells. TSG-6, a protein binding to HA, also forms a stable complex with a serum proteinase inhibitor IαI (Inter-α-inhibitor) with a synergistic effect on the plasmin-inhibitory activity of the latter. Plasmin is involved in the activation of the proteolytic cascade of matrix

metalloproteinases and other proteinases that lead to inflammatory tissue damage. Therefore, the action of the TSG-6 / IαI complex, which can be further organized by binding HA into the cell matrix, can serve as a powerful link for negative feedback to moderate inflammation, and stabilize granulation tissue as healing progresses[6].

Cell migration is necessary for the formation of granulation tissue[6]. The early stage of granulation tissue is dominated by extracellular matrix-rich hyaluronic acid, which is considered a suitable environment for cell migration in this transient wound matrix. The contribution of hyaluronic acid to cell migration can be attributed to its physicochemical properties as well as its direct interactions with cells. Hyaluronic acid provides an open hydrated matrix that facilitates cell migration, while the other role is aimed at migrating and controlling locomotive cell mechanisms that are mediated through specific interactions between HA and HA surface receptors. As previously discussed, the three major surface receptors for HA cells are CD44, RHAMM, and ICAM-1. RHAMM is more associated with cell migration. Forms bonds with several protein kinases associated with cell locomotion, for example, extracellular signal-regulated protein kinase (ERK), p125fak, and pp60c-src[11,12,13]. During fetal development, the migratory pathway through which nerve cells migrate is rich in hyaluronic acid[6]. HA is closely related to the process of cell migration into the granulation tissue matrix, and studies have shown that cell movement can be inhibited, at least in part, by degrading HA or blocking HA receptor involvement[14]. By providing dynamic cell strength, HA synthesis has also been shown to be associated with cell migration[15]. In essence, HA is synthesized in the plasma membrane and released directly into the cell environment[6]. This may contribute to hydrated microecology at the sites of synthesis and is necessary for cell migration by facilitating cell separation.

HA is a key component of the periodontal ligament and plays a number of important roles in cell regeneration, migration, and differentiation mediated by various HA binding proteins and cell-surface receptors such as CD44. This CD44 antigen is expressed in periodontal tissues, and interaction with HA-CD44 has been associated with periodontal ligament (PDL) cell proliferation and mineralization activities[16]. CD44 is a single-chain molecule composed of: N-terminal extracellular domain containing ligand binding sites, proximal membrane membrane, transmembrane segment, and cytoplasmic portion[17]. The molecular size of CD44 ranges from 80 to 150 kDa depending on the variable fusion of at least 11 of the 21 exons encoding CD44. Specifically, periodontal ligament cells express the isoforms CD44 and CD44H[16,18]. CD44 regulates proliferation and mineralization of cells in the periodontal ligament. Zeinab Al-Rekabi and colleagues[19] at In vivo study prove key scientific research, where recent results suggest strong evidence that HA affects contractility in periodontal ligament cells, which in turn causes these cells to move more slowly and migrate shorter distances. Given the full focus of this study, it states that CD44, hyaluronic acid, and T-lymphocytes stimulate feedback from cytokines, and suppressor T cells as well as B-growing cells, but most importantly activate the macrophage system. This would follow that hyaluronic acid has the role of a powerful anti-inflammatory mediator, which would show a better and faster reparative effect in soft and hard tissues. HA is a metabolite or diagnostic marker of inflammation in gingival puncture of the gingiva as well as an important factor in growth, development and repair of tissues. Based on current evidence in the literature, it is now known that along with mechanical therapy, the use of chemotherapeutic agents provide a better treatment strategy. The most common chemotherapeutic agents are antimicrobial and anti-inflammatory drugs. They are administered either systemically or locally. Topical antimicrobial agents for the treatment of periodontal disease include chlorhexidine, tetracyclines, and metronidazole.

Low molecular weight hyaluronic acid has a significant angiogenic effect, while, surprisingly, high molecular weight has the opposite effect. Hyaluronic acid is a major component of the extracellular matrix and plays a key role in tissue regeneration, inflammatory response, and angiogenesis, which are stages of skin wound repair. As of 2016, reviews evaluating the effect of promoting wound healing, however, show only limited evidence from clinical trials affecting burns, diabetic feet, or any skin surgery. In gel form, hyaluronic acid combines with water and swells, making it useful in skin treatments such as dermal filler for the treatment of facial wrinkles and lasting about 6 to 12 months, clinical treatment with regulatory approval[20].

Hyaluronic acid can be degraded by a family of enzymes called hyaluronidases. In humans, there are at least seven types of hyaluronidase-like enzymes, several of which are tumor suppressors. Degradation products of hyaluronic acid, oligosaccharides, and very low molecular weight hyaluronic acid show pro-angiogenic properties[21]. In addition, recent studies have shown that fragments of hyaluronic acid, rather than a high molecular weight parent molecule, can cause inflammatory reactions in macrophages and dendritic cells in tissue injury and skin transplantation. Hyaluronic acid can also be degraded by non-enzymatic reactions. These include acid and alkali hydrolysis, ultrasonic decomposition, thermal decomposition, and oxidant degradation[22].

Recent studies of regenerative surgical procedures show that reducing the bacterial load on the wound site may improve the clinical outcome of regenerative therapy. High concentrations of medium and low molecular weight hyaluronic acid have the greatest bacteriostatic effect, especially on Aggregatibacter actinomycetemcomitans, species of Prevotella oris phylococcus aureus most commonly found in oral gingival lesions and periodontal wounds. Clinical application of hyaluronic acid membranes, gels, and sponges during surgical therapy may reduce bacterial contamination at the surgical wound site, reducing the risk of postoperative infection and promoting more predictable regeneration[1].

Hyaluronic acid has also been reported to be antiadhesive and antimicrobial, which are two more beneficial characteristics for scaffold implantation at injury sites. As a hydrophilic natural polymer, its antimicrobial effects on common orthopedic pathogens such as Staphylococcus, β-hemolytic Streptococcus, Pseudomonas aeruginosa, Enterococcus, and others have been proven in various studies[23, 24, 25], although other research has presented contradictory results for specific bacteria such as S. mutans[26]. The anti-adhesive and antibacterial properties of HA have also been studied, indicating a concentration-dependent anti-adhesive effect on Staphylococcus aureus, Haemophilusinfluenzae, and Moraxella catarrhalis. The effect of hyaluronidase-producing bacteria was not considered, however, and the inhibition mechanisms behind the results are unclear. Thus, further investigation is needed, as noted by the authors. In fact, prior to any practical applications, the antibacterial effect on common pathogens present at surgical sites needs to be considered[26]. In addition, employing the carrying ability of hyaluronic acid to load antiseptics is a better way to prevent infection.

A study by Yi Xu et al.[27] concluded that there was no clinical or microbiological improvement achieved by the adjunctive use of Hyaluronan 0.2% gel when compared to mechanical debridement. However in this study, Hyaluronan 0.2% gel was applied only once a week for six weeks, a total of seven applications over a six-week period, compared to the recommended application level of three times daily for at least four to eight weeks. The absence of observed clinical improvements, contrary to other published studies, may indicate that the Hyaluronan levels used in this study were well below the optimum levels required to achieve a significant clinical improvement. Vanden Bogaerde[2] in a recent clinical report evaluated the clinical efficacy of esterified hyaluronic acid in the treatment of infrabony periodontal defects. The author concluded that application of hyaluronic acid seems a promising method for the treatment of infrabony defects by inducing a significant reduction in pocket depth and promoting gain in clinical attachment.

An interesting in vivo study with convincing evidence is provided by the report of Masako Fujioka-Kobayashi[28], together with his colleagues, who examines the effect of HA on the periodontal ligament cells - cell compatibility, proliferation, and differentiation. Both hyaluronic acid formations, necroslinked (HA ncl) and crosslinked (HA cl) HA showed high viability of periodontal ligament cells (greater than 90%) regardless of culture conditions. Furthermore, no significant difference was observed in mRNA levels and proteins in proinflammatory cytokines, including MMP_2 and IL-1. Both, diluted HA_ncl and HA_cl, significantly increased cell numbers compared to the controlled TCP samples at 3 and 5 days. HA_ncl and HA_cl in standard cell growth media significantly reduced ALP staining, COL_1 immunization, and early regulated early osteogenic differentiation, including $Runx_2$, COL_1, and OCN mRNA levels, compared with controls. When osteogenic differentiation medium (ODM) was added, interestingly, the expression of early osteogenic markers increased with demonstration of higher levels of COL_1 and ALP expression; especially in HA 1:10 diluted state. Late-stage osteogenic markers remained inhibited. All of this led to the general conclusion that non-cross-linked and interconnected HAs maintained high periodontal ligament cell viability, increased proliferation, and early osteogenic differentiation. However, HA was consistently associated with a significant reduction in late osteogenic differentiation of primary human PDL cells.

Andrew L Raines et al.[29] in his study demonstrated that the enhanced neovascularization observed in ablated marrows treated with low MW NaHY + DBM is further increased when accounted for the volume occupied by the DBM, indicating that the neovascularization induced by NaHY is further stimulated by the presence of DBM. Also in his experiment in rat tibial marrow ablation model, both high MW HA with demineralized bone matrix (DBM), and low MW HA with DBM, induced a significant increase in the blood vessel volume fraction when compared with empty defects, suggesting that HA promotes neovascularization.

Palatal and gingival fibroblasts play an important role in oral wound healing. The use of bioactive substances that influence their behavior and thus support oral soft tissue wound healing/regeneration is of major clinical interest. Therefore, the aim of the study on Maria B Asparuhova and her associates[30] was to investigate the specific role of two commercially available HA formulations in affecting oral fibroblast cell behavior including proliferation, migration, and wound healing-related gene expression. All these processes affect soft tissue wound

healing/ regeneration following reconstructive periodontal surgery. Our data demonstrates that both formulations of HA (a) are fully biocompatible and exert no negative effects on the viability of HPFs and HGFs; (b) are able to increase the proliferative and migratory abilities of both cell types; (c) trigger expression of COL_3A_1 and $TGFB_3$ genes characterizing scarless fetal wound healing; (d) upregulate the expression of genes encoding the growth factors PDGFB, FGF-2, and EGF, which are essential for the wound healing process; (e) induce proinflammatory cytokine gene expression, thus potentially initiating a cellular inflammatory response; and (f) affect MMP gene expression either directly (MMP_1 and 8) or indirectly (MMP_2 and 3), potentially through induction of proinflammatory cytokines, thus influencing ECM remodeling. Finally, (g) our data point on Akt, Erk1/2, and p38 as the signaling molecules by which the two HA preparations exert their effects on oral fibroblasts. Understanding the mechanisms whereby these HAs function may reveal how to intervene in the dynamic process of oral soft tissue regeneration with the aim to improve it. They concluded that, both HA formulations investigated in the current study, exert diverse positive effects on human palatal and gingival fibroblasts, two cell types involved in soft tissue regeneration following periodontal reconstructive therapies that utilize palatal connective tissue or free gingival grafts. The observed pro-proliferative, pro-migratory and pro-wound healing properties of the two HAs speak in favor of their clinical potential. However, both HA formulations are biocompatible and enhance the proliferative, migratory and wound healing properties of cell types involved in soft tissue wound healing, following regenerative periodontal surgery.

Jung-Ju Kim and his associates (31), in one experimental study on dogs, filled the sockets with mineralized bone (47.80% ± 6.60%) and bone marrow (50.47% ± 6.38%) in the control group, whereas corresponding values were 63.29% ± 9.78% and 34.73% ± 8.97% for the test group, respectively. There was a statistically significant difference between the groups. Reversal lines and a copious lineup of osteoblasts were observed in the middle and apical parts of the sockets in the test group. An infected socket shows delayed healing of the socket wound, and HA, because of its osteo-inductive, bacteriostatic, and anti-inflammatory properties, may improve bone formation and accelerate wound healing in infected sockets. In the end they concluded and suggested that the application of HA with ACS in compromised extraction sockets provides enhanced regenerative efficacy for bone healing compared with rhBMP-2.

Peisong Zhai[32] and associates, in their study concluded that HA derivates incorporated composite scaffolds have shown excellent potential for improving osteogenesis and mineralization, and also HA should be a promising tool in bone regeneration.

Alcântara and his colleagues[33], gave a clear message with their study which contains: after each tooth extraction, the empty alveola must be filled with 1% of hyaluronic acid in the form of a gel, which would accelerate the process of bone formation significantly faster, relative to leaving the alveola empty. This study evaluated the effects of hyaluronic acid (HA) on bone repair of human dental sockets. Thirty-two lower first premolars were extracted from 16 patients (2 per patient) for orthodontic reasons. Following the extractions, one socket was randomly filled with 1% HA gel, while the other was allowed to naturally fill with blood clot. After 30 and 90 days of surgery, patients underwent cone beam computed tomography. Five central orthoradial slices were captured from each socket. The gray intensity was measured in each image and results were reported as mean percentage of bone formation. The buccolingual alveolar ridge width was measured, and dimensional changes were compared between the postoperative intervals. The pattern of alveolar trabecular bone was evaluated through the fractal dimension. Treated sockets showed a higher percentage of bone formation and fractal dimension values (58.17% and 1.098, respectively) compared with controls (48.97% and 1.074, respectively) in the 30-day postoperative period ($p < 0.05$). After 90 days, there was no significant difference between groups; it was performed on patients in whom the treated alveoli with HA and without 90 days were measured with CBCT where the measured bone parts were proven.

Kumar and his colleagues[34], have focused their research on the effects of hyaluronic acid on root canal procedures in addition to the coronary artery bypass graft (CAF) procedure. In this study design, 10 patients with 20 sites of Miller I recession were treated and monitored for a period of 6 months. Experimental sites were treated with 0.2% HA gel and CAF while control sites were treated with CAF only. There is a significant change in the outer surface of the root (RD), no probing bleeding (PPD), clinical attachment level (CAL), and percentage of root coverage in the two groups compared to baseline, but there is no statistically significant difference between the parts of the root. HA and control points in relation to RD, PPD and CAL. Although there is no statistically significant difference, root coverage at HA sites appears to be clinically more stable than the CAF-treated control site after 24 weeks.

Engström et al.[35] 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.

The esthetic area is defined as the visible area during functioning and includes the anterior maxillary and mandibular teeth. Interdental "black triangles" were considered as the third most disliked and less attractive esthetic problem after caries and crown margins. The loss of gingival papillary height results in open gingival embrasures which leads to several problems in phonetics, esthetic concerns, and food impaction. This loss of interdental papillae (IDP) commonly known as "black holes" or "black triangles". The gingival black space is the distance from the cervical black space to the interproximal contact. Different surgical and nonsurgical approaches are proposed in the periodontal literature to provide satisfactory IDP reconstruction. Several nonsurgical and surgical techniques for the reconstruction of lost IDP remain elusive. The compromised blood supply, scarring, and trauma in thin gingival biotypes increase the failure risk. Interdental papillae have always been of interest for research, especially its reconstruction in the event of its collapse, so a clinical study by Shivani Singh and Kharidi LaxmanVandana[36] shows that the application of hyaluronic acid 5% at the base of the papilla and other clinical points is significantly good because it completely fills the interdental space for up to 8 months. The dependence of the duration of hyaluronic acid in the soft tissue primarily depends on the fitness of the surrounding soft and hard tissue. The use of modified stent for clinical improvement assessment of IDP is recommended. Further, long-term studies would throw more insight with this regard. Further histologic studies are required to assess the mechanism of hyaluronic action.

Araújo Nobre and his colleagues[9] compared the health status of the peri-implantitis complex during the period of osteointegration of implants for immediate function, using HA gels or chlorhexidine (CHX). The purpose of this study was to compare the health status of the peri-implant complex (hard and soft tissues surrounding the implant) during the healing period of immediate function implants, using HA or CHX gels in the patient's maintenance protocol. Both groups were followed up for 6 months, with clinical observations on the 10th day, 2 months, 4 months and 6 months post-surgically. During the course of the study, HA and CHX produced good results in maintaining a healthy peri-implant complex in immediate function implants for complete rehabilitations in the edentulous mandible. Statistically significant differences were found in favor of the HA group in the modified bleeding index on the second observation (P = 0.003). The difference was more marked in the axial implants placed in the fifth sextant (P = 0.05). Correlation coefficient between plaque and bleeding index revealed a potentially better result for CHX at 6months. They found a statistically significant lower modified bleeding index in group HA in combination with the control group treated with CHX. It can be said with certainty that it is good to use a combination treatment with the use of HA 0.2% gel in the first 2 months and 0.2% CHX from 2 to 6 months.

After thorough examination and evaluation of the persisting data we can summarize that there is an evidence of multifunctional benefits of hyaluronic acid in periodontology.

Hyaluronic acid and multifunctional roles in periodontics:

Topical application of subgingival hyaluronic acid gel can be used as an antimicrobial agent as an adjunct to scaling and root planning. These data suggest that a hyaluronan containing gel has a beneficial effect in the treatment of plaque-induced gingivitis[37]. Johannsen et al.[38], in their study evaluated the adjunctive effect of the local application of a hyaluronan gel to scaling and root planning in the treatment of chronic periodontitis. Twelve patients with chronic periodontitis were recruited to participate in a study with a split-mouth design and provided informed consent. Plaque formation and bleeding on probing were evaluated pre-treatment (baseline) and at 1, 4, and 12 weeks post-treatment. Probing depths and attachment levels were evaluated at baseline and at 12 weeks. The patients received full-mouth scaling and root planning. A hyaluronan gel was administered subgingivaly in the test sites at baseline and after 1 week. The results show a significant reduction in bleeding on probing scores and probing depths were observed in both groups at 12 weeks (P<0.05). Significantly lower bleeding on probing scores were observed in the hyaluronan group compared to control at 12 weeks (P<0.05). Mean probing depth reductions between baseline and 12 weeks were 1.0+/- 0.3 mm and 0.8 +/- 0.2 mm for the hyaluronan and control groups, respectively. The difference between the groups was statistically significant (P<0.05). The conclusion of the author and his colleges is that the local application of hyaluronan gel in conjunction with scaling and root planning may have a beneficial effect on the periodontal health in patients with chronic periodontitis. The clinical application of hyaluronic acid in gingivitis therapy, the author Alexander Pistorius[6] in his study with sixty non-smoking outpatients in good general condition, with

clinical signs of gingivitis, were included. Forty patients (HA group, 20 men, 20 women; age: 32.8 +/- 11.3 years) used a spray containing HA 5 times daily over a period of 1 week. The control group consisted of 20 patients (10 men, 10 women; age: 31.3 +/- 9.3 years). The clinical parameters DMF-T (decayed, missing, filled teeth) index, approximal plaque index, sulcus bleeding index, papilla bleeding index, and gingival crevicular fluid were measured at baseline (T_1), after 3 days (T_2), and after 7 days (T_3). The results show a reduction in the sulcus bleeding index of the HA group (T_1: 72.9 +/- 19.5%) to 50.3 +/- 21.1% was noted at T_2, and at T_3 the sulcus bleeding index was 40.7 +/- 23.0%. The papilla bleeding index values of the HA group were 1.6 at T_1, 1.0 at T_2, and 0.7 at T_3. The gingival crevicular fluid showed significant reductions in the HA group. At T_1 the recorded mean value was 16.3, at T_2 it was 11.8, and at T_3 it was 7.9. Only insignificant changes were observed in the respective indices of the control group. There were no significant alterations in the plaque values of either group throughout the study period. Conclusion: the results obtained by this study demonstrate that the topical application of an HA-containing preparation represents a potentially useful adjunct in the therapy of gingivitis, although its use does not diminish the need for plaque reduction as a primary therapeutic measure.

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[2]. Claar performed a lateral coverage of the augmentation followed by use of cross-linked HA in gel form, which was developed especially for GBR[39]. The principles of GBR applications[40, 41] 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.

Non-surgical treatment of peri-implant pockets. The results obtained in this study favor the adoption of non-surgical protocols. The fact that no significant differences were found between both groups supports the research hypothesis in the use of HA in the treatment of pockets up to 5 mm, and of CHX for the treatment of pockets up to 6 mm. this conclusion means that it is possible that the use of non-surgical therapy is effective, making it possible either to treat peri-implant pathologies with a simple protocol, or to prepare the site for surgical therapy in case of an unsuccessful treatment[8].

Peri-implant maintenance of immediate function implants. The objective of this study was to compare the health status of the peri-implant complex (hard and soft tissues surrounding the implant) during the healing period of immediate function implants, using HA or CHX gels in the patient's maintenance protocol. Thirty complete edentulous patients, with four immediate function Brånemark System implants placed in the mandible (total of 120 implants), were randomly assigned to two groups (HA and CHX) using only these two chemicals in their daily implant self-care. Both groups were followed up for 6 months, with clinical observations on the 10th day, 2 months, 4 months and 6 months post-surgically. In the course of the study, HA and CHX produced good results in maintaining a healthy peri-implant complex in immediate function implants for complete rehabilitations in the edentulous mandible. Statistically significant differences were found in favor of the HA group in the modified bleeding index on the second observation ($P = 0.003$). The difference was more marked in the axial implants placed in the fifth sextant ($P = 0.05$). Correlation coefficient between plaque and bleeding index revealed a potentially better result for CHX at 6 months. The findings point out the importance of a maintenance protocol in immediate function implants. Both chemicals are valid tools for implant maintenance. The authors suggest that it might be advantageous to administer HA in the first 2 months and CHX between 2 and 6 months (9).

As autologous cell hyaluronic acid graft gingival augmentation in mucogingival surgery. Seven sites from 6 patients were used in this study. Five patients (5 sites) needed gingival augmentation prior to prosthetic rehabilitation, and one patient (2 sites) needed augmentation because of pain during daily tooth brushing. Full-mouth plaque score (FMPS), full-mouth bleeding score (FMBS), probing depth (PD), and clinical attachment level (CAL) were recorded for the sites at baseline and 3 months after surgery. The amount of keratinized tissue (KT) was measured in the mesial, middle, and distal sites of each involved tooth. A small 2 x 1 x 1 mm portion of gingiva (epithelium and connective tissue) was removed from each patient, placed in a nutritional medium, and sent to the laboratory. The gingival tissue was processed: keratinocytes and fibroblasts were separated and only

fibroblasts were cultivated. They were cultured on a scaffold of fully esterified benzyl ester hyaluronic acid (HA) and returned to the periodontal office under sterile conditions. During the gingival augmentation procedure, the periosteum of the selected teeth was exposed, and the membrane containing cultivated fibroblasts was adapted to, and positioned on the site. Three months after surgery, an increased amount of gingiva was obtained, and the histological examination revealed a fully keratinized tissue on all the treated sites. Prato and his colleagues (10) concluded that tissue engineering technology using an autologous cell hyaluronic acid graft was applied in gingival augmentation procedures, and provides an increase of gingiva in a very short time without any discomfort for the patient.

As a carrier for newer molecules in various regenerative procedures. In one study, the osteoinductive effect of the hyaluronic acid (HA) by using an esterified low-molecular HA preparation (EHA) as a coadjuvant in the grafting processes to produce bone-like tissue in the presence of employing autologous bone obtained from intra-oral sites, to treat infra-bone defects without covering membrane, was examined. The report on 9 patients with periodontal defects treated by EHA and autologous grafting (4 males and 5 females, all non smokers, with a mean age of 43.8 years for females, 40.0 years for males and 42 years forthe group, in good health) with a mean depth of 8.3 mm of the infra-bone defects, as revealed by intra-operative probes. Data were obtained at baseline before treatment and after 10 days, and subsequently at 6, 9, and 24 months after treatment. Clinical results showed a mean gain in clinical attachment (gCAL) of 2.6mm of the treated sites, confirmed by radiographic evaluation. Such results, the author Andrea Ballini[42] suggested, that autologous bone combined with EHA seems to have good capabilities in accelerating new bone formation in the infra-bone defects.

As a biomaterial scaffold in tissue engineering research.

Hyaluronic acid is biocompatiable and intrinsically safe to use, with no evidence of cytotoxicity found[11]. Hyaluronic acid gel, injections or oral (by mouth), should not be used in patients with allergies.

Recent studies on regenerative surgical procedures indicate that reduction of bacterial burden at the wound site may improve the clinical outcome of regenerative therapy. The high concentration of medium and lower molecular weight hyaluronic acid has the greatest bacteriostatic effect, particularly on Aggregatibacter actinomycetemcomitans, Prevotella oris, phylococcus aureus strains commonly found in oral gingival lesions and periodontal wounds. Clinical application of hyaluronic acid membranes, gels and sponges during surgical therapy may reduce the bacterial contamination of surgical wound site, thereby, lessening the risk of postsurgical infection and promoting more predictable regeneration. Also, Hyaluronic acid may act as biomaterial scaffold for other molecules, such as BMP-2 and PDGF-BB, used in guided bone regeneration techniques and tissue engineering research[43].

Conclusion

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 acid-based 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. We urge that more attention should be paid to

controlled delivery as well as biomimetic scaffold and carrier designs, not just HA-based forms. In conclusion, hyaluronic acid has overwhelmingly proven its potential for use in bone regeneration and should be considered as a useful option in future applications.

Conflicts of interest

The authors confirm that this article content has no conflict of interest.

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