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PHD THESIS

**A CLINICAL AND HISTOLOGIC EVALUATION OF HYALURONIC
ACID IN PERIODONTAL DEFECT TREATMENTS AND PAPILLA
AUGMENTATION**

A B S T R A C T

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INTRODUCTION

Periodontal disease is an inflammatory condition affecting the periodontal tissues, leading to attachment loss, alveolar bone destruction, and ultimately, the loss of the affected teeth. According to the coronal or apical location of the base of the pocket in relation to the bone crest, bone loss after periodontal disease has either a suprabony or an infrabony pattern. In patients diagnosed with periodontitis, suprabony defects are highly common, whereas infrabony defects are slightly less prevalent. Nevertheless, if left untreated, intraosseous lesions pose a significant risk of further progression, ultimately resulting in tooth loss. Since the mid-1950s, various surgical techniques that preserve as much as possible periodontal tissues, followed by the additional use of various biomaterials have been developed. Together with basic/clinical research involving suprabony and

infrabony defects, they expanded periodontal surgical procedures to restore function and improve aesthetics.

Initially discovered and isolated by Karl Meyer and John Palmer in the 1930s, hyaluronic acid (HA) has a significant historical background. HA possesses unique properties, including its extensive water-binding capacity and high viscosity. By promoting extracellular matrix synthesis, cell proliferation, and wound healing, HA can reduce inflammation and restore tissue integrity. The anti-inflammatory properties of HA have been evidenced in several studies. HA's ability to reduce bacteria and inflammation makes it a promising local chemotherapeutic agent for periodontal disease.

In 2018, significant international advances were made in the field of periodontology by introducing the New Classification Scheme of Periodontal and Peri-implantation Diseases and Conditions and subsequently in the field of surgical periodontal treatment by publishing the S3 Level Clinical Practice Guideline for treatment. Ongoing efforts are being made to identify novel aids in periodontal treatment protocols and various biomaterials are being researched for this purpose. Hyaluronic acid products show great potential in assisting with the treatment of periodontal disease and the regeneration of lost periodontal structures.

A strong commitment to improving treatment protocols and diagnostic techniques for periodontal and peri-implant diseases characterizes the national and regional research direction of the Department of Periodontology at the Faculty of Dental Medicine of the Victor Babes University of Medicine and Pharmacy, Timisoara. This department has conducted rigorous studies to enhance conventional treatment methods for periodontal disease, peri-implantitis, and mucositis. The ongoing investigation of innovative and cost-effective therapies demonstrates this goal.

The thesis aims to thoroughly investigate the distinct attributes and efficacy of adjunctive hyaluronic acid products used in periodontal defects surgical treatments, which can be categorized into two primary types based on their position in relation to the alveolar bone: suprabony defects and infrabony defects. For this research, different studies were carried out in order to gather information and insights, which were then analyzed in order to determine the advantages of HA as an adjunct therapeutic agent for periodontal defect treatments. The objective was to comprehend HA's function and its potential advantages in improving periodontal treatment results. In addition, the purpose of this research was to use histological analysis in order to acquire a comprehensive understanding of the influence that HA has on the healing process of periodontal tissues. Analyzing tissue samples at different stages of the healing process allowed for the observation of the specific cellular and tissue-level changes induced by HA. This provided valuable insights into the biological effectiveness of HA in promoting periodontal regeneration and repair, thus enhancing our understanding of its therapeutic potential. Through structuring the doctoral research around these objectives, the studies collectively improved the understanding of HA's role in periodontal regeneration, optimize its use, and ensure the practical application of the findings in clinical settings.

The thesis begins with a list of published scientific papers, and an introduction that outlines the scope and significance of the research on periodontal regeneration treatments of suprabony and infrabony defects and its challenges.

The general part comprises of three chapters: the first addresses the patterns of bone loss in periodontal disease and the challenges in treating them, the second explores biomaterials used in periodontal regeneration focusing on enamel matrix derivatives (EMD) and HA, and the third reviews current concepts for periodontal non-surgical and surgical treatments with the additional use of HA, as well as for the treatment of gingival deformities. The first part of this thesis reflects the current state of knowledge in the field, summarized in the following paragraphs.

There are various patterns of bone loss encountered in patients with periodontal disease. Suprabony defects are the most prevalent type of bony defects and the most difficult to regenerate. Infrabony defects are less prevalent, and more susceptible to regeneration, however, specific conditions are needed for the success of the treatment.

Over the years a multitude of biologic materials have been used in the treatment of periodontal defects. EMD is a material used on an international scale that has proven impressive abilities to regenerate periodontal defects through various histological and clinical studies, on animal and human models. However, the material is considered expensive to use in current practice, and not all patients can afford its use for the treatment of defects caused by periodontal disease. Also, to have ideal results when using EMD, certain conditions must be met during surgical treatments according to the manufacturer's recommendations.

HA is a new material in the palette of biomaterials used in periodontal regeneration and papillae augmentation. Numerous *in vitro* and *in vivo*, clinical and histological, animal or human model studies have already brought to light the many positive properties of hyaluronic acid products in tissue healing. These products are safe for human use in non-surgical and surgical periodontal treatment with a low number of studies reporting minor adverse reactions, and the price of the product compared to that of EMD is much more affordable for general public use. In addition, these products are easy to use by practitioners and do not require special conditions to obtain positive results.

Based on the data provided, several questions emerged after conducting the literature review: "Is HA a reliable biomaterial in the surgical treatment of periodontal infrabony defects?", "What are the advantages of hyaluronic acid compared to EMD?", "How can HA improve the results after the surgical treatment of suprabony defects?", "What are the cellular changes that occur in the soft tissue following the injection of hyaluronic acid for papillary augmentation?"

The second part of the thesis (personal contribution) includes three studies (two clinical and one histological), aimed to answer the previously stated questions.

The first arm of the personal research is represented by a **randomized clinical trial** that assessed the adjunctive effects of cross-linked HA (xHyA) gel in the regenerative periodontal surgery of intrabony vertical defects from a clinical and radiographic perspective. This first randomized

prospective single-blind clinical study was conducted with a parallel design of two independent groups and a 1:1 allocation ratio, involving a total of 57 defects. In the test group, additional HA gel was used in the treatment of intrabony defects, while in the control group, EMD was used in the same treatment. Clinical attachment level (CAL) gain was considered the primary outcome, while pocket probing depth (PPD), gingival recession (REC), bleeding on probing (BOP), full-mouth plaque score (FMPS), full-mouth bleeding score (FMBS), and radiographic parameters such as defect depth (BC-BD), and defect width (DW) were considered secondary outcome variables. Clinical and radiographic outcomes were assessed at baseline and six months following the treatment in both groups, which underwent identical surgical procedures. The statistical analysis revealed significant improvements in the intragroup comparison, with a significant mean CAL gain observed in both groups ($p < 0.001$). Compared to 33.3% of control sites, 48.1% of test sites achieved CAL gains ≤ 2 mm. The mean PPD reduction obtained a statistically significant result in the intragroup comparison in both groups ($p < 0.001$). The mean REC increase was similar in both groups 1.04 ± 1.29 mm vs. 1.11 ± 1.22 mm (test vs. control). The mean BC-BD, DW, FMPS, FMBS, and BOP obtained statistically significant results only in the intragroup comparison and not the intergroup comparison. The results support HA products as a suitable and easy to use treatment alternative for the treatment of periodontal intrabony defects.

The second component of the personal research involves a **randomized clinical trial** that evaluated the additional benefits of applying HA gel in the treatment of periodontal suprabony defects. The second study is the first to assess the additional benefits of HA in the treatment of suprabony periodontal defects and was structured as a randomized, controlled, double arm (Universities of Naples and Timisoara), double-blind clinical trial. In the test group, the suprabony defects were surgically treated with open flap debridement (OFD) and an xHyA gel application, while in the control group, they were treated using OFD alone. Results from 60 patients were statistically analyzed at baseline and 12 months following treatment. The main outcome variable was CAL while the secondary outcome variables were changes in mean PPD, gingival recession (GR), FMPS, and FMBS. Results reported a statistically significant difference in the mean CAL gain ($p < 0.001$) in the intergroup comparison [3.06 ± 1.13 mm (test) vs. 1.44 ± 1.07 mm (control)]. PPD reduction of test group measurements (3.28 ± 1.14 mm) versus the control group measurements (2.61 ± 1.22 mm) were statistically significant ($p = 0.032$). GR changes were statistically significant only in the test group 0.74 ± 1.03 mm ($p < 0.001$). FMBS and FMPS revealed a statistically significant improvement mostly in the test group. These findings provide further support for the efficacy of HA products in treating periodontal suprabony defects. The study suggests that additional use of HA products can be a viable substitute for the conventional surgical treatment (OFD and resective osseous surgery) for this condition.

The third component of the personal research is represented by a **human histologic study** that evaluates the early healing process following papilla augmentation with injectable HA gel. The third human histologic study was designed as an experimental study and is the first to assess the early healing in three time points after a papilla augmentation procedure with injectable HA. Fifteen papillae

from two patients with stage III, grade B periodontitis have been selected for this study. Every week for three weeks, five papillae were injected once with HA gel, and during the fourth week, the papillae were surgically removed as part of step 3 of the periodontal treatment. The histological analysis was performed on fifteen papillae, with five papillae corresponding to every time point of healing (weeks 1, 2, and 3). The primary outcome was considered the newly formed collagen fibers. The presence of residual HA, the integrity of epithelium or the presence of erosions/ulcerations, the presence and characteristics of inflammatory infiltrate, the presence of granulomatous reactions, and interstitial edema were considered secondary outcomes. The results indicated that from the second to the third week, newly formed connective tissue begins to appear, while the observed HA pools (vesicles) content decreases. The density of inflammatory infiltrate was higher in the first week after injection, decreasing considerably by week 3; however, it was still visible throughout the healing time points. A granulomatous reaction was present in only three samples, while no signs of ulceration or necrosis could be observed; however, epithelial erosions could be observed on some samples after the first week. The study concluded that fibroblast activity does not seem to be impaired, despite the concurrent presence of residual HA vesicles and a foreign body granulomatous reaction, suggesting once again that HA is a biomaterial that aids the healing process of periodontal tissues.

The present research concludes with a summary of findings, personal contributions, and a bibliography.

CONCLUSIONS AND PERSONAL CONTRIBUTIONS

The first arm of the present research is represented by a clinical trial on the regeneration of intrabony periodontal defects using HA. This study provided a rigorous comparison between xHyA and EMD, contributed valuable insights into HA efficacy, and offered improvements in clinical protocols. By demonstrating that xHyA can achieve similar results to EMD, this research supports the adoption of xHyA as a simpler, cost-effective, and equally effective treatment alternative for intrabony periodontal defects. To our knowledge, this is the first study to compare HA with EMD in the surgical treatment of intrabony defects using OFD as a flap technique.

The second arm of the present research is represented by a clinical trial on the treatment of suprabony periodontal defects using HA. This study explored whether applying xHyA gel can provide additional clinical benefits when combined with OFD. The positive results of this study are paving the way for incorporating xHyA into standard surgical protocols, thus improving patient outcomes and expanding the toolkit available to periodontists for managing suprabony defects. To the best of our knowledge, this is the first study to use HA as an adjunctive biomaterial in the surgical treatment of suprabony periodontal defects.

The third arm of the present research is represented by a human histologic study on the evaluation of early healing after papilla augmentation procedures with HA. This study was motivated by

the need to understand the biological interactions between HA and human tissues at the cellular level during the early phases of healing. By conducting a detailed histological analysis of the healing process following papilla augmentation with HA, at different time-points this study aimed to elucidate the patterns of collagen fiber formation, inflammatory responses, and epithelial integrity. The findings from this study provided foundational knowledge that supports the clinical use of HA in papilla augmentation, highlighting its benefits and lack of limitations. Ultimately, this research can lead to improved strategies for managing periodontal soft tissue deficiencies, contributing to better aesthetic and functional outcomes for patients. To the best of our knowledge, this is the first human histologic study to evaluate early healing after HA injection.

The first scientific objectives of the doctoral research was to determine the clinical effectiveness of xHyA gel and to compare it with EMD in improving clinical and radiographical measurements after the surgical treatment of intrabony vertical defects. Concerning this objective, my findings indicated that after 6 months, CAL as the primary outcome clinical parameter obtained statistically significant differences in the intragroup comparison. However, no difference was observed in the intergroup comparison suggesting that HA is an effective alternative for EMD in the treatment of infrabony defects. This statement is sustained further by similar results observed after the statistical analysis of the secondary clinical parameters such as PPD, REC, FMPS, FMBS, early healing index (EHI), and the radiographic parameters BC-BD and DW. No adverse reactions have been reported during this clinical trial, thus promoting xHyA as a safe biomaterial to be used in surgical periodontal regeneration of intrabony defects.

The second scientific object was to evaluate the additional clinical benefits of applying HA gel in the treatment of periodontal suprabony defects. Concerning this objective my findings indicated that after 12 months, the primary outcome parameter, CAL, obtained a statistically significant difference in the intergroup analysis. The same pattern was also observed for the rest of the investigated parameters PPD, GR, FMPS, and FMBS. These results suggest that HA is a valuable biomaterial as an adjunctive in the treatment of suprabony periodontal defects. No adverse reactions have been reported by the participants emphasizing the safety of the biomaterial used.

The third scientific objective was to investigate the early histologic healing process following injection with HA gel at several time points. Concerning this third objective my findings indicated that new collagen fibers, regarded as the primary outcome could be observed from the second week of healing. The presence of residual HA, inflammatory infiltrate, and interstitial oedema regarded as secondary outcomes, could also be observed in all samples. These findings suggest that although inflammation is present within the tissues after injection, HA promotes new collagen fiber formation and does not impair fibroblast activity, thus aiding the regeneration process of the periodontal structures. None of the patients reported adverse reactions thus further establishing the safety of the material in dental use.

As a result of the previously mentioned studies some advantages of the investigated product and treatment protocols can be detailed.

The first and foremost advantage of the HA gel product investigated in the three studies that make up the present research is the clinical efficiency observed after statistically analyzing the clinical, radiographical, and histological parameters. The first clinical trial established HA as a suitable product for the treatment of infrabony defects. After statistically analyzing the clinical parameters obtained from both the test (HA) and control group (EMD) similar results were obtained for both groups in the intragroup analysis, with no statistically significant difference in the intergroup analysis, thus promoting the HA as an equivalent alternative for EMD in periodontal infrabony defect treatments. Similar positive results for the test group (OFD+HA) were obtained by the second study that compared OFD with (test group) or without the additional use of HA (control group) in the periodontal treatments of suprabony defects. All measured parameters obtained a statistically significant difference in the test group for the intragroup and intergroup analysis. Last but not least the third human histologic study revealed that new collagen fibers appear from the second week of healing after HA gel injection, regardless of the inflammatory infiltrate presence suggesting once again that HA has the potential to aid in the regeneration of periodontal tissues.

The HA product investigated; a cross-linked HA gel (hyaDentBG®) has proved to be a safe biomaterial. No adverse reactions were recorded during the human histologic trial and the two clinical trials. As such HA can successfully be used in the periodontal regeneration of infrabony and suprabony defects as well as in papilla augmentation procedures.

Another advantage of the investigated material is its cost-effectiveness. The proposed xHyA biomaterial is significantly more affordable compared to the “golden standard” EMD treatment, obtaining the same results in the treatment of infrabony defects as observed from the first clinical trial of the present research. Moreover, in the treatment of suprabony defects, the material obtained better results compared to the standard treatment of these defects as observed from the second clinical trial of this research making it an accessible alternative that can be used by healthcare systems.

Ease of use is another advantage of the HA product. The product tested in the three studies that make up the special part of the present research is a class III medical device in the form of cross-linked HA gel named hyaDent BG®, which is presented as an injectable gel in 1.2 ml cartridges. For its use, a Uniject syringe can be employed making the product a user-friendly device. According to the manufacturer, the presence of blood does not impair its properties, making it a suitable product especially useful in surgical periodontal procedures as compared to other biomaterials (e.g. EMD) by shortening the surgical time.

Some disadvantages could also be noted, and the first one is that for the treatment of infrabony defects using HA specific requirements of the treated defects are needed (e.g. 3 wall defects).

Another disadvantage of the HA tested product is the gel-like form, although better than a liquid form, for periodontal regeneration a more solid form of HA is considered a better alternative for maintaining space and allowing tissues to regenerate.

In order to comprehend the role of HA in tissue regeneration, future research should focus on the specific molecular and cellular pathways through which HA exerts its effects on periodontal tissues. Additionally, the interaction of HA with growth factors, cytokines, and extracellular matrix components should also be analyzed.

Innovations in minimally invasive methods of delivering HA to periodontal tissues to improve its efficacy in various periodontal conditions, as well as the development of new HA formulations with sustained release to offer continuous therapeutic effects over long periods, are of utmost importance for future research direction.

Future research directions worth pursuing include evaluating the long-term outcomes of HA treatment in various periodontal conditions, such as peri-implantitis and mucositis, and conducting large-scale, randomized controlled trials comparing HA with other regenerative therapies to establish its relative efficacy and safety.

The biocompatibility of new HA formulations and delivery systems in both *in vitro* and *in vivo* models, as well as the monitoring of adverse events and complications associated with repeated or prolonged use of HA, are additional future research directions. These efforts will ensure that HA-based therapies do not induce cytotoxic effects or adverse immune responses.

Ultimately, the improvement of patient outcomes and the advancement of periodontal therapy can be achieved by addressing these future research directions and enhancing the understanding of hyaluronic acid's role in periodontal regeneration and clinical applications.

The importance of the research lies in its potential to enhance the treatment protocols for intrabony vertical defects, the pioneering evaluation of HA in the treatment of suprabony horizontal defects, two common and challenging periodontal conditions, and also the pioneering nature of histological assessment of human early wound healing after HA papilla augmentation procedures.