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The potential of hyaluronic acid in the treatment of periodontal inflammation

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"Trabalho apresentado à Universidade Fernando Pessoa

como parte dos requisitos para obtenção do grau

de Mestre em Medicina Dentária"

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(Cécile Bousquet)

Abstract

Hyaluronic acid is a naturally occurring linear polysaccharide identified in all periodontal tissues in varying quantities. It is a very promising alternative as a mediator of periodontal tissue regeneration, because of its vast presence in periodontal tissues and its anti-inflammatory capacity. The objective of this literature review was to evaluate the potential of hyaluronic acid in the treatment of periodontal inflammation.

A survey was conducted between November 2016 and March 2017 in *MEDLINE, B-on, Scopus and Google Scholar database*. Manuscript analysis was substantiated on Meta-Analysis Reporting Standards. The languages elected for articles selection were English, French, Portuguese and Spanish, and the time limit was the period from 2007 to 2017. That search resulted in 33 articles with the eligibility criteria.

Hyaluronic acid has shown anti-inflammatory, anti-oedematous and anti-bacterial effects in the treatment of periodontal diseases. In the treatment of gingivitis or periodontitis, hyaluronic acid presents advantages when used alone or used as an adjuvant.

Despite hyaluronic acid identified properties, further long-term studies need to be carried out discussing application time, quantity of application, different forms and concentrations. These studies could also optimize administration protocols and establish proper recommendations for its use.

Hyaluronic acid presents beneficial effects on periodontal inflammation, with importance on a microbiological level with favorable clinical outcomes.

Key words: hyaluronic acid, gingival inflammation, periodontal disease, periodontal regeneration.

Resumo

O ácido hialurónico é um polissacarídeo linear naturalmente identificado em todos os tecidos periodontais, em quantidades variadas. É uma alternativa muito promissora como mediador da regeneração periodontal, devido à sua vasta presença nos tecidos periodontais e à sua capacidade anti-inflamatória.

O objetivo deste trabalho de revisão bibliográfica foi avaliar o potencial do ácido hialurónico no tratamento da inflamação periodontal.

Foi realizada uma pesquisa bibliográfica entre novembro de 2016 e março de 2017 nas bases de dados *MEDLINE, B-on, Scopus e Google Scholar*. A análise de cada artigo fundamentou-se nos critérios de meta-análise: Meta-Analysis Reporting Standards. Os idiomas destacados para a seleção dos artigos foram: inglês, francês, português e espanhol. O período de revisão foi de 2007 a 2017. Essa pesquisa resultou em 33 artigos com os critérios de elegibilidade.

O ácido hialurónico tem demonstrado efeito anti-inflamatório, anti-edematoso e anti-bacteriano no tratamento da doença periodontal. No tratamento da gengivite ou periodontite o ácido hialurónico apresenta vantagens quando usado isoladamente ou utilizado como adjuvante.

Apesar das propriedades comprovadas do ácido hialurónico, mais estudos deverão ser realizados discutindo o tempo de aplicação, quantidade de aplicação, diferentes formas de apresentação e concentrações a utilizar. Estes estudos favorecerão uma melhor compreensão do efeito terapêutico do acido hialurónico permitindo optimizar os protocolos de administração e estabelecer recomendações adequadas para o seu uso.

Comprova-se que o ácido hialurónico tem efeitos benéficos sobre a inflamação periodontal, com importância a nível microbiológico e por isso com resultados clínicos favoráveis.

Palavras-chaves: Ácido hialurônico, inflamação gengival, doença periodontal, regeneração periodontal.

Dedications

Je remercie tout d'abord mes parents qui m'ont inspiré et grâce à qui j'ai pu suivre cette voie, j'espère vous rendre fiers,

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GENERAL INDEX

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Abbreviations and acronyms

β - Beta

BOP - Bleeding on probing

CAL - Clinical attachment level

CD14 - Cluster of differentiation 14

CD44 - Cluster of differentiation 44

CHX - Chlorhexidine

ECM - Extracellular matrix

GAG - Glycosaminoglycans

GBI - Gingival bleeding index

GI - Gingival index

HA - Hyaluronic acid

HAS - Hyaluronan synthase

LPS - Lipopolysaccharide

MMPs - Matrix metalloproteases

NOD - Nucleotide-binding oligomerization domain

PAL - Probing attachment level

PAMPs - Pathogens-Associated Molecular Patterns

PBI - Papilla bleeding index

PGE-2 - Prostaglandins 2

PI - Plaque index

PMNs - Polymorphonuclear leukocytes

PPD - Probing pocket depth

PRRs - Pattern Recognition Receptors

SRP - Scaling and Root Planning

I. INTRODUCTION

The periodontium is a set of specialized tissues that both surround and support the teeth. Those tissues show a particular conformation in witch non-mineralized and mineralized tissues coexist in a perfect symbiosis, and work like a barrier against microbial invasion which is essential to prevent the destruction of periodontal tissues (alveolar bone, cementum, and periodontal ligament). Indeed, the crossing of this obstacle by microbial agents can lead to chronical inflammation, such as periodontal disease (Gontiya G, Galgali SR, 2012).
Periodontal diseases are a common inflammatory disorder known as gingivitis and periodontitis. They result in the destruction of the tissues surrounding and supporting the teeth, leading to tooth loss (Muhammad Ashraf Nazir, 2017).

Hyaluronic acid (HA) is a high molecular weight polysaccharide (glycosaminoglycan), which plays a vital role in the functioning of extracellular matrices, including those of mineralized and non-mineralized periodontal tissues (Vera RN *et al.,* 2013).

It has already been used in the treatment of the inflammatory process in various domains such as orthopedics, dermatology and ophthalmology. In dentistry, it played a role in the temporomandibular joint disorders, and more recently in the treatment of periodontal disease thanks to its anti-inflammatory, anti-oedematous and anti-bacterial effects (Parveen D, Reet K, 2013).

With the realisation of this work of literature review, we attempted to evaluate the involvement of HA in the periodontal regulation of inflammation and periodontal regeneration. So the aim of this study was to know if HA presents good results in the treatment of periodontal inflammation.

A survey was conducted between November 2016 and March 2017 in *MEDLINE, B-on, Scopus and Google Scholar database*, using the following terms in different combinations: *hyaluronic acid, gingival inflammation, periodontal disease, periodontal regeneration.* Manuscript analysis was substantiated on Meta-Analysis Reporting Standards. Only articles in English, French, Portuguese and Spanish, and from 2007 to 2017 were selected. That search resulted in 33 articles with the eligibility criteria. The journal “Periodontology 2000” has been also used as a complementary help for this review.

II. THEORETICAL FRAMEWORK

1. Periodontal disease

1.1 Definition and classification

Periodontal diseases usually refer to common inflammatory disorders known as gingivitis and periodontitis which are caused by a pathogenic microbial infiltration of the periodontal tissues. This infiltration leads to a microscopically and clinically visible inflammation and immunological reactions (Nora Silva *et al.,* 2015).

Indeed, those bacteria induce the production of cytokines and chemokines by the gingival epithelium, resulting in the expression of adhesion molecules, increased permeability of gingival capillaries and attraction of polimorphonuclear neutrophils through the junctional epithelium and into the gingival sulcus. Then the inflammation spread deep into the tissues and causes loss of supporting connective tissue, and alveolar bone (Ford PJ *et al.,* 2000).

Various classifications of periodontal diseases were born during the years and replaced by new ones to improve our understanding of the etiology and pathology of periodontal diseases, and to provide better periodontal treatments. Also in order to facilitate professionals communication, and systematize information. The latest classification of periodontal diseases proposed by Gary Armitage in 1999 is presented in appendix 1.

Gingivitis is an inflammatory lesion localized in the superficial periodontium, that means the gingival epithelium and the underlying connective tissue, without affecting the deep periodontal structures. Gingivitis is a reversible disease. Clinical signs of gingivitis include change of color, shape and texture of the gingiva (sign of inflammation), spontaneous or induced bleeding, localized pain and the appearance of false pockets of a gingival hypertrophy, without apical migration of the epithelial attachment (American Academy of Periodontology, 2017).

Chronic periodontitis is an inflammatory and infectious diseases of all tissues supporting the teeth, leading to the progressive destruction of deep periodontal tissues. They are irreversible and are accompanied by migration of the junctional epithelium along the root of the tooth, leading to the appearance of periodontal pockets and gingival recessions, which are clinical signs of this affection (American Academy of Periodontology, 2017).

1.2 Etiology

Periodontal health resides on the balance between all the elements of the periodontium and the ambient factors. If this equilibrium is not respected it can lead to periodontal disease. They are two types of risk factors, the modifiable ones and the non-modifiable ones, modifiable risk factors are usually environmental or behavioural in nature whereas the non-modifiable risk factors are intrinsic to the individual (Muhammad Ashraf Nazir, 2017).

The responsible for the initiation of periodontal diseases are bacterial species living in the biofilms present on the gingiva or below the gingival margin, and they progress thanks to the inflammation initiated by specific subgingival species (Ricardo Teles *et al.,*2013).
In the microflora of gingivitis, bacteria gram-positives coexist (*Actinomyces naeslundii*, *Streptococcus sanguis*, *Fusobacterium nucleatum*), present in healthy periodontal tissues, and bacteria gram-negatives (*Tannerela forsythia*, *Treponema denticola,* among others) which number increase with the progression of gingivitis (Lockart PB *et al*., 2012).

The principal bacteria associated with the condition of periodontitis are *T. forsythia, P. gingivalis,* e *T. denticola,* called « the red complex », due to the vast factors of virulence that they present, leading to an inflammatory and immunological response of the host (Dentino *et al*., 2013).

Despite the fact that bacteria are the principal etiological factor of gingivitis and periodontitis, this microbial aggression is not sufficient to induce periodontal disease. Indeed, the pathogenesis of periodontal diseases is complex and the susceptibility to it varies between individuals (Van Dyke TE, Sheilesh D, 2005).

Kazor *et al.* (*cit. in* Genco e Borgnakke 2013) says that tobacco seems to increase the number of specific periodontal pathogenic agents such as *P. gingivalis, T denticola* and *T. forsythia.* Smoking also modify the host response to the challenge of bacteria in microbial dental plaque (Shchipkova AY *et al.,* 2010).

Diabetes is a modifiable factor because though it cannot be cured, it can be controlled. Studies have shown a relationship between poor glycemic control and periodontal disease parameters (Guzman *et al.,* 2003; Tsai *et al.*, 2002).

Stress has been linked, by many studies, with the development of clinical attachment loss and loss of alveolar bone (Hugoson *et al.,* 2002; Pistorius *et al.,* 2002; Wimmer *et al.,* 2002). Maybe the relationship is due to the fact that individuals under stress are less likely to perform regular good oral hygiene and prophylaxis (Croucher *et al.,* 1997).

Regarding genetic risk factor, studies conducted on twins suggest that 50% of susceptibility to periodontal disease is due to host factors (Michalowicz *et al.,* 2000). It appears that a genetic polymorphism is linked to a greater risk of developing periodontitis (Dentino *et al.*, 2013).

In osteoporosis, the low density present in the maxillary and mandibular bones causes an increase of porosity, alterations in the bone trabeculae pattern, and may lead to a greater reabsorption of the alveolar bone after the infection by the pathogens of the periodontium (Stabholz *et al.,* 2010).

Recent studies have suggested that obesity is associated with oral diseases, and particularly periodontitis (Dalla Vecchia CF *et al.,* 2005). In fact, the adipose tissue secretes several cytokines and hormones that are involved in inflammatory processes, who could lead to tissue destruction of the periodontium (Dentino *et al*., 2013).

Studies suggest that alcohol consumption is associated with moderately increased severity of periodontal disease. It's a dose-dependency perspective, so the higher the alcohol consumption, the greater the severity of the loss of clinical insertion (Genco and Borgnakke, 2013).

Finally, gender is also considered a risk factor. For years, it has been described that men of different ethnicities, geographical locations and of different ages have more periodontal disease than women (Genco and Borgnakke, 2013).

1.3 Pathogenesis

In 2008 Kornman reviewed the model of the pathogenesis of periodontal disease. He says that human periodontitis is initiated and perpetuated by a small group of predominantly gram-negative, anaerobic or microaerophilic bacteria that colonize the subgingival area, but that host factors are

essentials for the disease to occur and progress.

Bacteria first triggers an innate inflammatory response, which is the first line of defense of the host, and is able to recognize those invading microorganisms as non-self. Microorganisms do this invasion through antigens, lipopolysaccharide (LPS) and other virulence factors, the host respond by releasing antibodies and leukocytes (PMNs), more precisely neutrophils. This innate response also results in the release of mast cells, macrophages, T and B lymphocytes and plasma cells (Page and Kornman, 1997; Sallum *et al.,* 2004).

This initial line of defense will activate the release of pro-inflammatory cytokines, namely IL-1, IL-6, IL-8 and TNFα who will signal fibroblasts to produce prostaglandins (PGE-2) and Matrix metalloproteases (MMPs). PGE-2 appears to be involved in bone destruction and MMPs in the destruction of connective tissue (Tülay YL and Tove B, 2013).

So bacteria mostly cause the observed tissue destruction indirectly, and they do so by activating various components of the host defense systems in such a manner that destruction happens (Souza, J.A *et al.,* 2012).

2. Inflammation and regeneration in the periodontal disease

2.1 Host defense mechanisms

Periodontal inflammation is characterized by the appearance of vascular and cellular changes leading to temporary or permanent deterioration of the constituents of normal tissues (cells, fibers, matrix). This results in alteration or loss of the normal function of the affected tissue. The main objective of this local inflammatory reaction is to protect the exposed tissue against the penetration of harmful substances and to establish favorable conditions for the regeneration or repair of damaged tissue structures (Hasturk H *et al.,* 2015).

Most of the microorganisms invading the periodontium have virulence factors capable of causing massive tissue destruction both directly, through tissue invasion and the production of virulent substances, or indirectly, by activation of host defense mechanisms, creating a powerful

inflammatory infiltrate that can interfere with normal host defense mechanisms. In response to this aggression, the host activates an innate immune response which is improved by an adaptive immune response leading to an efficient microbial clearance (Bascones-Martinez A *et al.,* 2009).

The first line of defense, the innate system, is triggered by Pattern Recognition Receptors (PRRs) that bind Pathogens-Associated Molecular Patterns (PAMPs). These receptors include toll-like receptors, nucleotide-binding oligomerization domain (NOD) proteins, cluster of differentiation 14 (CD14), complement receptor-3, lectins and scavenger receptors (Takeda K*, et al.,* 2001).
The studies are mainly focused on toll-like receptors who recognize a large number of varied and complex pathogen-associated molecular patterns, and on neutrophils who are one of the first-responders of inflammatory cells to migrate toward the site of periodontal inflammation. Polymorphonuclear neutrophils are the hallmark of acute inflammation. Although they are protective cells, their accumulation and massive death are the cause of tissue breakdown in progressive periodontitis (Van Dyke TE, 2007).
Adaptive immunity cells and specific cytokines have been described as important players in the periodontal reaction to invaders, with a special attention to CD4+ T-cells (T-helper cells) (Cutler CW, Jotwani R, 2004).

Despite all those protective systems, the persistent interaction between periodontal inflammation and bacterial infection up-regulates the expression and activity of neutral proteinases which contributes to the progressive breakdown of periodontal supporting tissue. Thus, the excessive host inflammatory response and/or inadequate resolution of inflammation seems to be critical to the pathogenesis of periodontal disease (Sorsa T, *et al.*, 2011).

2.2 Biological principles of periodontal regeneration

Regeneration is the biological process by which the architecture and function of tissues injured during a pathological process are fully restored. It is the *ad-integrum* reconstruction of the attachment apparatus, cementum, ligament and alveolar bone around a tooth whose periodontium has been injured (American Academy of Periodontology, 2001).

An essential goal for intervention in inflammatory disease is the return of tissue to homeostasis. That is to say, the rapid and complete elimination of invading leukocytes from a lesion which is the ideal outcome following an inflammatory event (Van Dyke TE., 2007).

At the end of the inflammatory process when there is a high concentration of proinflammatory products, such as prostaglandin E_2 (PGE_2), a "class switch" may occur within neutrophils. This leads to the synthesis of proresolving molecules through distinct pathways from those involved in the generation of proinflammatory lipid mediators. Those proresolving molecules include lipoxins that control the resolution phase of acute inflammation and promote healing of the lesion (Levy BD, *et al.,* 2001).

Others proresolving molecules such as resolvins and protectins have been discovered, and represent a potentially powerful intervention that stimulates resolution pathways leading to the restoration of homeostasis. They are capable of limiting polymorphonuclear neutrophil (PMN) migration into sites of inflammation, activating monocytes, and stimulating the uptake of apoptotic PMNs by macrophages (Serhan CN and Chiang N, 2008).

However, with a defective immune response and the incapacity to return to homeostasis, periodontal inflammation can become chronic and can result in a prolonged inflammatory reaction and host tissue damage (Hasturk H *et al.*, 2015).

2.3 Factors that influence the healing process

Periodontal healing is a complex biological process that consists of hemostasis, inflammation, proliferation, and remodelling. Single or multiple factors may play a role in one or more of those phases, contributing to the overall outcome of the healing process (Guo S and DiPietro LA, 2010).

An increased age is a major risk factor for healing process. Indeed, it is recognized that the effect of aging causes a temporal delay in periodontal regeneration, mostly because of an altered inflammatory response (Swift *et al*., 2001).

Sex hormones play a role too in periodontal regeneration. Indeed, estrogen affects healing by regulating a variety of genes associated with regeneration, and the genes primarily associated with inflammation (Hardman and Ashcroft, 2008).

The pathophysiology of stress results in the deregulation of the immune system. Thus, psychological stress impairs normal cell-mediated immunity, causing a significant delay in the healing process (Godbout and Glaser, 2006).

Diabetic individuals exhibit a documented impairment in the healing of acute inflammation. Several dysregulated cellular functions are involved, these defects are responsible for inadequate bacterial clearance and delayed or impaired repair (Loots *et al.,* 1998; Sibbald and Woo, 2008).

Many medications, such as those which interfere with clot formation or platelet function, or inflammatory responses and cell proliferation have a significant impact on healing (Guo S and DiPietro LA, 2010).

In obese persons, adipokines, coming from adipocytes, have a profound and negative impact on the immune and inflammatory response which seems to influence the healing process (Calabro and Yeh, 2007; Wozniak *et al.,* 2009).

Studies have demonstrated effects of alcohol on host-defense mechanisms, that results in suppressed pro-inflammatory cytokine release in response to an inflammatory challenge (Greiffenstein and Molina, 2008).

Tobacco smoke seems to be affecting several cell types and processes that are important to healing (Ahn *et al*., 2008).

Nutrition is also important in the process of healing. For example, vitamin C deficiencies leads to an impaired immune response and impaired healing (Campos *et al*., 2008).

3. Hyaluronic acid: its potential as a complement in the periodontal therapy

3.1 Presentation of hyaluronic acid

Hyaluronic acid (HA) was discovered and named in 1934 in the Department of Ophthalmology of the University of Colombia, by Mr Karl Meyer and Mr John Palmer. It is a polysaccharide isolated from the vitreous humor of the eye which contains a "non-sulfated" uronic acid bound to a hexosamine (Parveen D, Reet K, 2013). Since the international nomenclature of polysaccharides of 1984, it is also called "hyaluronan" (Nusgens BV, 2010).

HA belongs to the family of glycosaminoglycans (GAG), indeed, it is constituted by repeating units of d-glucoronic acid and N-acetyl-d-glucosamine connected by a glycosidic bond β(1-3) (Laurent TC, Fraser JR, 1992).

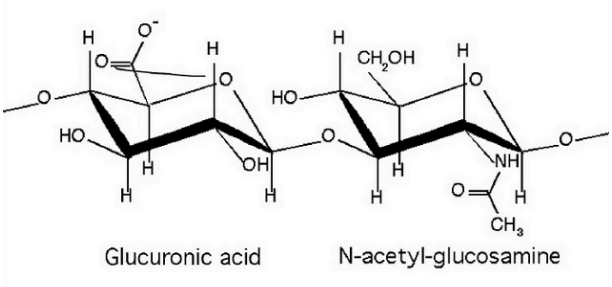

Fig.1: Chemical structure of HA (J. Mater and Chem. B, 2017)

HA is found in all the organism, and especially in the extracellular matrix (ECM) of soft connective tissues (Laurent TC, Fraser JR, 1996). It has been identified in all periodontal tissues in varying quantities, being more prominent in the non-mineralized tissues compared to mineralized tissues (Vera RN *et al*., 2013). It is a key element in both tissue structure, cell signaling and tissue hemostasis. It regulates the cell-matrix and cell-cell exchanges as well as the entry and exit of nutrients and waste, all in physiological and pathological conditions (Vigetti D, *et al.,* 2014).

The production of intrinsic HA happens thanks to hyaluronan synthase (HAS) enzymes (HAS1, HAS2, and HAS3) in various cells from the periodontal tissues including fibroblasts, keratinocytes, cementoblasts, and osteoblasts (Ijuin C, Ohno S, *et al.,* 2001).

Prehm's study in 1984 showed that HA is synthesized on the internal face of the plasma membrane of the cell, and then directly secreted into the extracellular space. Then its turnover occurs by

lymphatic drainage to the blood stream or by local metabolism involving specific enzymes named hyaluronidases. Those enzymes will permit to split the HA chains in small simple sugars that will be reusable in various metabolic cycles (Vigetti D, *et al.*, 2014).

Once AH has been synthesized, the molecules can be free in the extracellular space or remain attached to the plasma membrane by the HAS or via their specific receptor CD44, to form a layer of pericellular HA (Auvinen P *et al*., 2014).

Released into the extracellular space, AH fragments are incorporated into the ECM and are capable of interacting via receptors with other components of the ECM to transmit different cellular signals and thereby influence the behavior of the cells, thus acting on their differentiation, migration, etc. (Itano N, 2008). Indeed, HA can have cell surface interactions and bind with transmembrane glycoproteins called "hyaladherins". For example, the receptor CD44 discovered by Underhill *et al.* (Sherman L, *et al.,* 1994) or RHAMM which mediates mobility discovered by Turley (Turley EA *et al.,* 1991).
When HA remains attached to the plasma membrane it forms a pericellular mantle ("glycocalyx") which permits for example the bonding of the ECM, or the regulation of cell proliferation and migration, but also the protection of cells against viruses or cytotoxic (Itano N, 2008).

HA has two essential functions, a structural role because it is a constituent of the architecture of the tissues that increase the volume of the ECM by filling containing water and ions, and a regulating role of cell signalization because it interacts with constituents of the MEC and with membrane receptors (Vabres P, 2010).

3.2 Applicability in periodontics

Our knowledge of the mechanism of inflammation and healing process associated with periodontal disease showed us the potential of the components of the extracellular matrix as promoters of periodontal tissue regeneration and healing. Various studies supporting the role of those matrix components, highlighted hyaluronic acid as one possible candidate in regeneration of periodontal tissues (Vera RN *et al.,* 2013).

Indeed, preliminary clinical trials conducted by Vangelisti and Pagnacco *et al.* in 1997 have shown that HA has anti-inflammatory, anti-edematous and anti-bacterial effects for the treatment of gingivitis and periodontitis. It plays an anti-inflammatory role through the inhibition of tissue destruction and facilitates healing, because it reduces prostaglandins, metalloproteinases and other bio-active molecules (Laurent TC *et al.,* 1996).

HA has numerous roles in the initial inflammatory stages, such as improved inflammatory cell infiltration into the inflammatory site, in order to speed up the gingival immune response. Indeed, Hakansson *et al*. said that HA has a role in migration and adherence of polymorphonuclear leukocytes and macrophages at the inflamed site, and phagocytosis and destruction of microbial pathogens. So HA directly prevents proliferation of anaerobic pathogenic bacteria. It also indirectly acts to moderate inflammation and stabilize the granulation tissue by preventing degradation of the ECM proteins by enzymes-protease of inflamed cells (Parveen D, Reet K, 2013).

HA is also useful during granulation phase as it promotes cell proliferation, migration of matrix cells into granulation tissue matrix and granulation tissue organization (Bartold PM, Page RC, 1986). In later stage of the granulation phase, HA synthesis ceases and existing HA is cut into molecules of lower molecular weight by hyaluronidases which leads to an alteration in the composition of the granulation tissue. Those small fragments of HA promote the formation of blood vessels (angiogenesis) within wound sites (Vera RN *et al.,* 2013).

Hyaluronic acid is a key component of chronic injuries during wound healing processes in periodontal tissues, mostly in the processes of inflammation, granulation tissue formation and restauration of the epithelium (Nora Silva et al., 2015).

3.3 Applicability of HA as an adjuvant in the treatment of periodontal disease

To treat oral diseases chemical usage is widely employed, and various antibiotics and anti-inflammatory agents have been trailed in previous studies. However, recent initiatives have started

to use chemotherapeutic agents as a treatment of periodontal diseases, and HA is a recent addition to those (Sapna N, Vandana KL, 2011).
In fact, HA is interesting using as an exogenous agent in the treatment of chronic inflammatory changes due to his role in the control of mechanisms of inflammation and tissue regeneration. Because it's non-toxic, biocompatible, and it has numerous biochemical and physio-chemical features, its topical and systemic application offers a lot of benefit effects in the regulation of the host response (Vera RN *et al.,* 2013).

The treatment of gingivitis requires the teaching of oral hygiene techniques that permits plaque control, and the cleaning of teeth surfaces to remove plaque and tartar. However after scaling, agents can be used locally by patients at home to maintain periodontal health (Caton J., 1989).

Several studies have been conducted to study the efficacy of HA in the treatment of gingivitis. Sahayata in 2014 has showed that although scaling is effective in the treatment of gingivitis, its effect is enhanced by HA which reduces bleeding and flowing of the gingival fluid which acts as a microbial reservoir. In addition, he confirms that the topical use of HA as an anti-inflammatory agent improves significantly the clinical symptoms of the gingivitis.

In his study of 2005 Pistorius evaluated the results of application of HA spray on patients with gingivitis, and those results have shown a significantly reduced papillary bleeding index (PBI) in the group having used this HA spray compared to the placebo group.

The proprieties of HA also seem to be an asset for the treatment of periodontitis.
Indeed, in 2002 Mesa has shown a beneficial effect of the use of HA alone, without any other associated periodontal treatment, on the inflammatory character of periodontitis. The results show a reduction of the inflammatory infiltrate, accompanied by a reduction in the gingival bleeding index (GBI).

Scaling and Root Planning (SRP) is the first-line treatment for periodontitis with pockets and consists of stopping the inflammatory process by destabilizing and removing the pathogenic gingival biofilm and restoring an environment compatible with periodontal health (Heitz-Mayfield LJA, 2005). However, the effectiveness of SRP is dependent on the type of lesion and its access,

so topical application of subgingival HA gel can be used as an antimicrobial agent as an adjunct to SRP to overcome those limitations (Johannsen A *et al.*, 2009).

Bevilacqua in his study of 2012 suggested that subgingival application of HA following ultrasonic mechanical instrumentation is beneficial for improving periodontal parameters, and reducing bleeding and probing depth.

Polepalle in 2015 showed a significant improvement in all clinical gingival parameters, GBI and gingival inflammation, and periodontal parameters, pocket depth and clinical attachment gain, following subgingival application of a gel 0.8% HA in addition to root debridement (SRP). As well as a bacteriostatic effect on certain pathogens.
This beneficial effect of HA on gingival inflammation is also observed by Pilloni (2011) when used as an adjunct to mechanical home plaque control. And Gontiya (2012), who confirms the efficacy of SRP and concludes that HA improves clinical gingival parameters and helps prevent the progression of periodontal disease.
Johannsen *et al.* (2009) evaluated the adjunctive effect of local application of 0.8% Hyaluronan gel to SRP in the treatment of chronic periodontitis and found out in addition to a statistically different decrease in the bleeding index, a pocket depth significantly decreased when hyaluronic acid is used.

In addition, Eick (2012) concluded that HA stabilizes low rates of periodontal microbial flora, and prevents the re-growth of certain bacteria.

III. DISCUSSION

The question that served of fundament for the elaboration of this work was: “What are the potential effects of HA in the treatment of periodontal inflammation?”
We have highlighted the fact that HA has anti-inflammatory, anti-edematous and bacteriostatic effects. Indeed, almost all studies show that HA has a strong interest in the treatment of periodontal disease and more precisely in periodontal inflammation.

Sahayata in 2014 pursued a study about the application of a 0,2% HA gel (Gengigel®) in the treatment of gingivitis after scaling compared to scaling alone. He found a statistical significant amelioration on the gingival index (GI) and the PBI in the HA group compared to the placebo group, but no microbiological improvement of inflammation.

Two studies compared the efficacy of a HA gel applied after regular tooth brushing with regular tooth brushing alone (Pilloni *et al.,* 2011, Mesa *et al.,* 2002). They both concluded to a significant improvement of inflammation and bleeding, the following clinical indexes were improved: Bleeding on probing (BOP), Probing pocket depth (PPD), and GI.

Four studies compared the outcomes of the application of a HA gel after SRP compared to the application of a placebo gel after SRP (Bevilacqua *et al.,* 2012, Rajan *et al.,* 2014, Gontiya *et al.,* 2012, Polepalle *et al.,* 2015). The first two noticed a clinical improvement in the HA group with a significant amelioration in PPD and BOP, but Rajan (2014) noticed also an amelioration on the clinical attachment level (CAL).
The results of the study of Gontiya (2012) showed an improvement of the gingival parameters in the HA group but not of the periodontal ones, indeed he noticed a significant amelioration in GI and GBI. He also analyzed the inflammatory infiltrate but did not found any significant differences between the two groups.
On the contrary, Polepalle (2015) found a significant difference for the inflammatory infiltrate between the HA group and the placebo group. He noticed a significant improvement in BOP and PPD in the HA group, like Bevilacqua (2012) and Rajan (2014), but also in the plaque index (PI) for which he is the only one of all those studies.

The results from the previous studies are opposed to Xu who, a few years earlier (2004), did not conclude an improvement of periodontal health following the use of HA in complement of a SRP (no clinical or microbiological improvement). But in this study the HA gel was only applied once a week for 6 weeks which is not enough compared to the recommended application level of 3 times per day for at least 4 to 8 weeks, and the levels of HA were well below optimum levels required to achieve a significant clinical improvement (Sahayata 2014).

Despite the fact that HA gel is a good adjuvant to periodontal disease treatment, it's interesting to know if it has additional effects compared to other exogenous agents such as chlorhexidine (CHX). In his study in 2013, Chauhan compared the outcomes of a subgingival application of a HA gel after SRP, a CHX gel after SRP and SRP alone. After 3 months, there was a better amelioration of PPD and CAL in the HA group but it was non-significant compared to the CHX group, as well for the microbiological markers of inflammation. For some authors the CHX remains a reference agent in the treatment of periodontal disease but they suggest to use HA as an alternative to CHX in case of allergy or side effects. Further studies are needed in this field to better understand this topic (Chauhan AS, *et al.* 2013).

IV. CONCLUSION

HA is a natural component of our body and is abundant in the periodontal tissue, thus it can be used in humans without causing an immune or inflammatory response.
It has viscoelastic properties which permits to act like a barrier against periodontal pathogens, and make it practical to use in the form of a gel, allowing an easy application.
In addition, it has a proangiogenic, anti-inflammatory, and bacteriostatic role which influence positively the periodontal inflammation and healing when used alone or used as an adjuvant.
Indeed, the majority of the studies exposed in this review of literature confirm that HA used as an adjuvant associated with scaling seems to obtain better results in the treatment of gingivitis than isolated scaling.
In periodontitis, the combined treatment HA + SRP, presents more satisfactory results than the conventional treatment of SRP alone, in most of the outcome variables presented.
All this suggests that hyaluronic acid is an adjuvant of choice in periodontal therapy.

However, in order to simplify its use, studies still need to be carried out to specify the methods of administration and to establish proper recommendations. Thus, its use by general practitioners would be easier, more common and would improve the results of their periodontal treatments, leading to periodontal health and aesthetics in accordance with the patient's demand.

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Cécile Bousquet

The potential of hyaluronic acid in the treatment of periodontal inflammation

APPENDICES

Universidade Fernando Pessoa

Faculdade de Ciências da Saúde

Porto, 2017

APPENDIX 1

Classification of periodontal disease, Workshop 1999 (Armitage GC 1999)

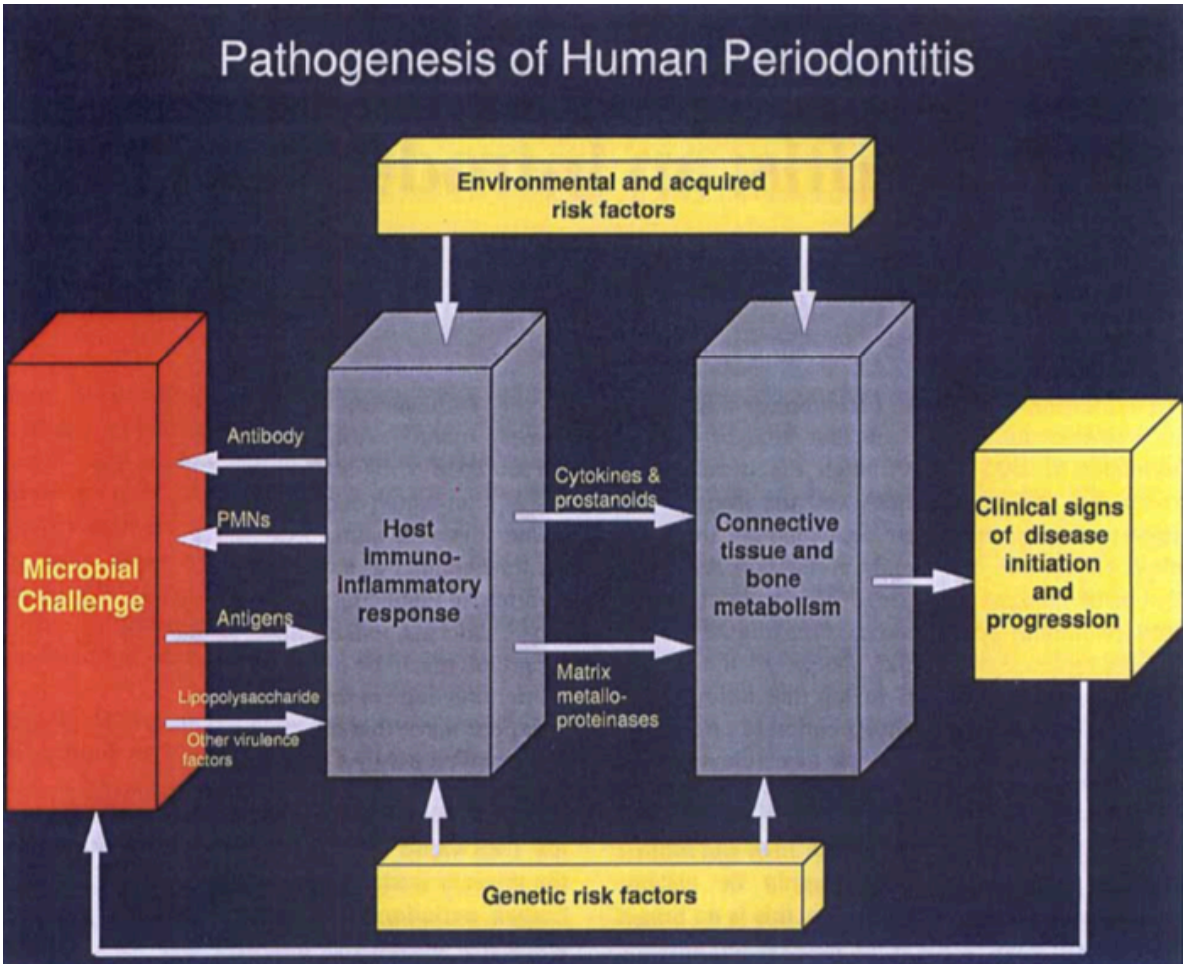
Appendix 1. Classification of periodontal disease, Workshop 1999 (Armitage GC 1999)

Gingival disease Dental plaque-induced gingival diseases Gingivitis associated with dental plaque only Gingival disease modified by systemic factors Gingival disease modified by medications Gingival disease modified by malnutrition Non-plaque-induced gingival diseases Gingival disease of specific bacterial origin Gingival disease of viral origin Gingival disease fungal origin Gingival disease of genetic origin Gingival manifestations of systemic conditions Traumatic lesions Foreign body reactions Not otherwise specified	**Necrotizing periodontal disease** Necrotizing ulcerative gingivitis Necrotizing ulcerative periodontitis
Chronic periodontitis Localized Generalized	**Abscesses of the periodontium** Gingival abscesses Periodontal abscesses Pericoronal abscesses
Aggressive periodontitis Localized Generalized	**Periodontitis associated with endodontic lesions** Combined periodontic-endodontic lesions
Periodontitis as a manifestation of systemic diseases Associated with hematologic disorders Associated with genetic disorders Not otherwise specified	**Developmental of acquired deformities and conditions** Localized tooth-related factors that modify or predispose to plaque-induced gingival diseases/periodontitis Mucogingival deformities and conditions around teeth Mucogingival deformities and conditions on edentulous ridges Occlusal trauma

APPENDIX 2

The pathogenesis of human periodontitis (Roy C. Page & Kenneth S. Kornman, 1997)

Appendix 2. The pathogenesis of human periodontitis (Roy C. Page & Kenneth S. Kornman, 1997)



APPENDIX 3

Cytokines and periodontal disease (Journal of Applied Oral Science, 2015)

Appendix 3. Cytokines and periodontal disease (Journal of Applied Oral Science, 2015)