

SCIENTIFIC EVIDENCE OF
CLEAN&SEAL
FOR PERIODONTAL AND
PERI-IMPLANT INFECTIONS

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Overview of Publications

CLEAN&SEAL	PERISOLV	SODIUM HYPOCHLORITE	HYADENT BG	HYALURONIC ACID
Diehl et al.2022	Guarnelli et al2015	Bevilacqua et al 2012	Shirakata et al 2022	Jimbo et al. 2018
	Iorio-Siciliano et al 2021	Bürgers et al 2012	Shirakata et al 2021	Pirnazar et al. 1999
	Jurczyk et al 2016	Galván et al 2014	Shirakata et al 2021	King et al 1991
	Mayer et al 2020	Gosau et al 2009	Bozic et al. 2021	Sasaki et al 1995
	Radulescu et al 2022	Gottardi et al 2010	Pilloni et al 2021	Aya et al 2014
	Schmidlin et al. 2017	Gottardi et al 2013	Eliezer et al 2019	Fawzi et al 2012
		Kalkwarf et al 1982	Eliezer et al 2018	
		Mohammadi et al 2008	Asparuhova et al 2019	
			Asparuhova et al. 2020	
			Guldener et al 2021	
			Lanzrein et al 2021	
			Pilloni et al 2018	
			Fujioka-Kobayashi et al 2017	
			Müller et al. 2016	

Relevant Studies on **PERISOLV** and **hyaDENT BG**

Diehl, D.; Friedmann, A.; Liedloff, P.; Jung, R.M.; Sculean, A.; Bilhan, H. Adjunctive Application of Hyaluronic Acid in Combination with a Sodium Hypochlorite Gel for Non-Surgical Treatment of Residual Pockets Reduces the Need for Periodontal Surgery—Retrospective Analysis of a Clinical Case Series. *Materials* 2022, 15, 6508. <https://doi.org/10.3390/ma15196508>

Abstract

The comprehensive treatment of periodontitis stage 2 to 4 aims at the resolution of periodontal inflammation and “pocket closure”, which implies a residual probing depth of ≤ 4 mm and a negative BoP. However, supportive periodontal therapy (SPT) regularly leaves behind persistent periodontal pockets with 5 or more mm in residual PPD and sites that often re-colonize and re-infect. Various adjunctive options for subgingival instrumentation have been proposed to enhance the antimicrobial effects to better control the re-infection of these residual sites. The locally applied adjuncts, based on their anti-inflammatory effect, are sodium hypochlorite antiseptic cleaning gel and cross-linked hyaluronic acid (xHyA). Both recently moved into the focus of clinical research on non-surgical and surgical therapy for periodontitis. The surgical use of xHyA indicates regenerative potential, supporting periodontal regeneration. This case series retrospectively analyzes the clinical benefits of the consecutive flapless application of sodium-hypochlorite-based cleaning gel and xHyA at the SPT to achieve pocket closure, thereby reducing the need for periodontal surgery. In 29 patients, 111 sites received the treatment sequence. At 6-month re-evaluation, an overall PPD reduction exceeding 2 mm was achieved, associated with a similar CAL gain (2.02 mm); the bleeding tendency (BoP) was reduced by >60%. Pocket closure occurred in almost 25% of all the sites. Within their limits, the present data suggest that the proposed **combined adjunctive treatment of residual active periodontal sites yielded significant improvement in the clinical parameters**. Further studies in RCT format are required to confirm these observations.

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<https://www.mdpi.com/1996-1944/15/19/6508/pdf>

Relevant Studies on **PERISOLV**

Guarnelli ME, Vecchiatini R, Farina R. 'Professional local administration of chloramine-based treatment in conjunction with ultrasonic mechanical instrumentation: clinical outcomes in patients with deep periodontal pockets persisting following active non-surgical therapy' *Minerva Stomatologia*, 2015; 64 suppl.1(2): 158-159

Background

Recently, a chemical agent (Perisolv®) has been proposed as an adjuvant to non-surgical therapy. The rationale for its use is based on the bactericidal effect of chloramines and a softening effect on debris that must be mechanically removed from the root surface.

Objective

To evaluate the clinical outcomes of ultrasonic mechanical instrumentation (UMI) when performed in conjunction with local application of Perisolv® at deep periodontal pockets persisting following active, non-surgical therapy (NST).

Methodology

Three periodontitis patients showing residual sites positive to bleeding upon probing (BoP) with pocket probing depth (PPD) > 5 mm following NST were included. At randomly selected, BoP-positive sites with PPD > 5 mm, the activated agent was applied and left in place for 30 seconds before proceeding with full-mouth UMI. After 20-25 minutes from agent activation, the sequence (Perisolv® application + UMI) was repeated.

Results

Mean PPD was 5.7 ± 1.0 mm at the conclusion of NST, and decreased to 3.4 ± 0.5 mm at 4-6 weeks following the administration of UMI+ Perisolv®. All sites treated with UMI+ Perisolv® showed PPD ≤ 4 mm and were BoP-negative following treatment.

Conclusion

The professional, local administration of a chloramine-based treatment (Perisolv®) in conjunction with UMI resulted in a substantial improvement of the periodontal conditions of patients with deep periodontal pockets persisting following NST.

Iorio-Siciliano V, Ramaglia L, Isola G, Blasi A, Salvi GE, Sculean A. Changes in clinical parameters following adjunctive local sodium hypochlorite gel in minimally invasive nonsurgical therapy (MINST) of periodontal pockets: a 6-month randomized controlled clinical trial. *Clin Oral Investig*. 2021 Sep;25(9):5331-5340. doi: 10.1007/s00784-021-03841-8. Epub 2021 Mar 9. PMID: 33687555; PMCID: PMC8370947.

Background

The mechanical disruption and removal of the subgingival biofilm represent the most important step in the treatment of periodontitis. However, in deep periodontal pockets, mechanical removal of the subgingival biofilm is difficult and frequently incomplete. Preliminary findings indicate that the use of amino acid buffered sodium hypochlorite (NaOCl) gel may chemically destroy the bacterial biofilm and facilitate its mechanical removal.

Objectives

To clinically evaluate the efficacy of minimally invasive nonsurgical therapy (MINST) of periodontal pockets with or without local application of an amino acid buffered sodium hypochlorite (NaOCl) gel.

Materials and method

Forty untreated patients diagnosed with severe/advanced periodontitis (i.e. stage III/IV) with a slow/moderate rate of progression (i.e. grade A/B) were randomly allocated in two treatment groups. In the test group, the periodontal pockets were treated by means of MINST and NaOCl gel application, while in the control group, treatment consisted of MINST alone. Full-mouth plaque scores (FMPS), full-mouth bleeding scores (FMBS), probing depths (PD), clinical attachment levels (CAL) and gingival recessions (GR) were assessed at baseline and at 6 months following therapy. The primary outcome variable was PD reduction at sites with PD \geq 5 mm at baseline.

Results

At 6 months, statistically significant differences between the two groups were found ($p = 0.001$) in terms of PD and CAL change. No statistically significant differences were found in terms of GR ($p = 0.81$). The number of sites with PD \geq 5 mm and BOP (+) decreased statistically significantly ($p = 0.001$), i.e. from 85.3 to 2.2% in the test group and from 81.6 to 7.3% in the control group, respectively. Statistically significant differences between test and control groups were recorded at 6 months ($p = 0.001$). MINST + NaOCl compared to MINST alone decreased statistically significantly ($p = 0.001$) the probability of residual PDs \geq 5 mm with BOP- (14.5% vs 18.3%) and BOP+ (2.2% vs. 7.2%).

Conclusion

Within their limits, the present results indicate that (a) the use of MINST may represent a clinically valuable approach for nonsurgical therapy and (b) the application of NaOCl gel in conjunction with MINST may additionally improve the clinical outcomes compared to the use of MINST alone.

Clinical relevance

In patients with untreated periodontitis, treatment of deep pockets by means of MINST in conjunction with a NaOCl gel may represent a valuable approach to additionally improve the clinical outcomes obtained with MINST alone.

Jurczyk K, Nietzsche S, Ender C, Sculean A, Eick S. In-vitro activity of sodium-hypochlorite gel on bacteria associated with periodontitis. *Clin Oral Investig*. 2016 Nov;20(8):2165-2173. doi: 10.1007/s00784-016-1711-9. Epub 2016 Jan 12. PMID: 26759339.

Objectives

Sodium hypochlorite formulation (NaOCl gel), its components sodium hypochlorite (NaOCl), and the activating vehicle were compared with 0.1 % chlorhexidine digluconate (CHX) solution. The antimicrobial activity was proven by determination of minimal inhibitory concentrations (MIC), minimal bactericidal concentrations, and killing assays. Furthermore, the influence on formation as well as on a 4-day-old 6-species biofilm was tested.

Materials and method

Except for one strain (*Parvimonas micra* ATCC 33270 in case of NaOCl gel), the MICs both of the CHX solution and NaOCl gel did not exceed 10 % of the formulations' concentration. In general, MICs of the NaOCl gel were equal as of the CHX solution against Gram-negatives but higher against Gram-positive bacteria. CHX but not NaOCl gel clearly inhibited biofilm formation; however, the activity of NaOCl gel was more remarkable on a 4-day-old biofilm. NaOCl killed bacteria in the biofilm and interfered with the matrix.

Results

Except for one strain (*Parvimonas micra* ATCC 33270 in case of NaOCl gel), the MICs both of the CHX solution and NaOCl gel did not exceed 10 % of the formulations' concentration. In general, MICs of the NaOCl gel were equal as of the CHX solution against Gram-negatives but higher against Gram-positive bacteria. CHX but not NaOCl gel clearly inhibited biofilm formation; however, the activity of NaOCl gel was more remarkable on a 4-day-old biofilm. NaOCl killed bacteria in the biofilm and interfered with the matrix.

Conclusion

The NaOCl gel acts antimicrobial in particular against Gram-negative species associated with periodontitis. Moreover, its component NaOCl hypochlorite is able to alter biofilm matrices.

Clinical relevance

The NaOCl gel may represent a potential alternative for adjunctive topical antimicrobial treatment in periodontitis.

Mayer Y, Ginesin O, Horwitz J. A nonsurgical treatment of peri-implantitis using mechanic, antiseptic and anti-inflammatory treatment: 1 year follow-up. Clin Exp Dent Res. 2020 Aug;6(4):478-485. doi: 10.1002/cre2.286. Epub 2020 Mar 17. PMID: 32185910; PMCID: PMC7453777.

Objectives

The study's aim was to assess the clinical outcome 6 and 12 months after a nonsurgical treatment of peri-implantitis per se or in conjunction with a combination of local antiseptic and anti-inflammatory treatment.

Materials and method

Included were 69 patients with periodontitis, with 106 implants, diagnosed with peri-implantitis. Peri-implantitis was defined as radiographic bone loss ≥ 3 mm, probing depth (PD) ≥ 6 mm, with bleeding on probing. Group M peri-implantitis was treated with ultrasonic debridement and soft tissue curettage. Group P had additional implant surface treatment with rotatory hand piece composed of chitosan bristle, soft tissue curettage combined with application of 0.95% hypochlorite and 1 mg minocycline HCl.

Results

After 6 months, both groups demonstrated significant reduction of mean plaque index, PD, and clinical attachment level (0.71 ± 0.57 , 0.81 ± 0.55 ; 4.77 ± 0.73 mm, 4.42 ± 0.5 mm; 5.03 ± 0.86 mm, 5.13 ± 0.73 mm; respectively) and bleeding on probing. After 6 and 12 months, group P showed significantly better PD results compared to group M. The bleeding was significantly less in group P after 12 months ($15.3\% \pm 6.2$, $25.1\% \pm 8.2$, respectively).

Conclusion

Adjunctive treatment with local antiseptic and anti-inflammatories during mechanical phase was positively associated with inflammation reduction and connective tissue reattachment.

Radulescu V, Boariu MI, Rusu D, Roman A, Surlin P, Voicu A, Didilescu AC, Jentsch H, Siciliano VI, Ramaglia L, Vela O, Kardaras G, Sculean A, Stratul SI. Clinical and microbiological effects of a single application of sodium hypochlorite gel during subgingival re-instrumentation: a triple-blind randomized placebo-controlled clinical trial. *Clin Oral Investig*. 2022 Nov;26(11):6639-6652. doi: 10.1007/s00784-022-04618-3. Epub 2022 Jul 12. PMID: 35829773.

Objectives

The aim of this study is to assess the clinical and microbiological effects of a single subgingival administration of sodium hypochlorite gel (NaOCl) and compare it with 1% chlorhexidine (CHX) gel and a placebo gel following mechanical re-instrumentation during supportive periodontal therapy (SPT).

Materials and method

Sixty-two patients who had been treated for stage III-IV periodontitis and enrolled in SPT were included in the study based on following criteria: (1) active periodontal therapy completed at least 6 months before enrollment in the study, (2) presence of at least 4 non-adjacent sites with probing pocket depths (PPDs) ≥ 4 mm with bleeding on probing (BOP), or presence of 5-8 mm PPDs with or without BOP. All sites presenting PPD ≥ 4 mm and BOP at baseline and 3-, 6-, and 9-month follow-up timepoints were subgingivally re-instrumented with ultrasounds. Selected patients were randomly assigned into three groups and treated additionally with a single subgingival administration of NaOCl gel (group A); 1% CHX gel (group B); and placebo gel (group C). Main outcome variable was pocket closure at 12 months. Secondary outcome variables were changes in mean PPD, BOP, and clinical attachment level (CAL) along with changes in the numbers of the following five keystone bacterial pathogens: *Aggregatibacter actinomycetemcomitans* (A.a.), *Porphyromonas gingivalis* (P.g.), *Prevotella intermedia* (P.i.), *Tannerella forsythia* (T.f.), and *Treponema denticola* (T.d.).

Results

At 12 months, pocket closure was obtained in 77.5% in the NaOCl treated sites. The reduction in PPD was higher with CHX than with NaOCl, although a statistically significant adjunctive effect for NaOCl ($P = 0.028$) was only observed in comparison with placebo only. Mean CAL improved in all groups and at all timepoints, compared to the baseline ($P < 0.05$). However, after 6 months, CAL gain was statistically significantly higher in the NaOCl treated group than following application of CHX ($P = 0.0026$).

Conclusion

In SPT patients, a single adjunctive use of a NaOCl gel may provide benefits in controlling inflammation and residual pockets.

Clinical relevance

A baseline single application of NaOCl gel in conjunction with mechanical debridement may achieve substantial pocket closure in patients enrolled in SPT; treatment time, cost, and applicability considerations should be taken into account when selecting this therapy.

Schmidlin PR, Fujioka-Kobayashi M, Mueller HD, Sculean A, Lussi A, Miron RJ. Effects of air polishing and an amino acid buffered hypochlorite solution to dentin surfaces and periodontal ligament cell survival, attachment, and spreading. *Clin Oral Investig*. 2017 Jun;21(5):1589-1598. doi: 10.1007/s00784-016-1950-9. Epub 2016 Sep 5. PMID: 27596604.

Objectives

The aim of this study is to examine morphological changes of dentin surfaces following air polishing or amino acid buffered hypochlorite solution application and to assess their influence on periodontal ligament (PDL) cell survival, attachment, and spreading to dentin discs in vitro.

Materials and method

Bovine dentin discs were treated with either (i) Classic, (ii) Plus, or (iii) Perio powder (EMS). Furthermore, Perisolv® a hypochlorite solution buffered with various amino acids was investigated. Untreated dentin discs served as controls. Morphological changes to dentin discs were assessed using scanning electron microscopy (SEM). Human PDL cells were seeded onto the respectively treated discs, and samples were then investigated for PDL cell survival, attachment, and spreading using a live/dead assay, adhesion assay, and SEM imaging, respectively.

Results

Both control and Perisolv®-rinsed dentin discs demonstrated smooth surfaces at low and high magnifications. The Classic powders demonstrated the thickest coating followed by the Powder Plus. The Perio powder demonstrated marked alterations of dentin discs by revealing the potential to open dentinal tubules even before rinsing. Seeding of PDL cells demonstrated an almost 100 % survival rate on all samples demonstrating very high biocompatibility for all materials. Significantly higher PDL cell numbers were observed on samples treated with the Perio powder and the Perisolv® solution (approximately 40 % more cells; $p < 0.05$). SEM imaging revealed the potential for PDL cells to attach and spread on all surfaces.

Conclusion

The results from the present study demonstrate that cell survival and spreading of PDL cells on root surfaces is possible following either air polishing or application with Perisolv®. Future in vitro and animal testing is necessary to further characterize the beneficial effects of either system in a clinical setting.

Relevant Studies on Sodium Hypochlorite

Bevilacqua L, Eriani J, Serroni I, Liani G, Borelli V, Castronovo G, Di Lenarda R. Effectiveness of adjunctive subgingival administration of amino acids and sodium hyaluronate gel on clinical and immunological parameters in the treatment of chronic periodontitis. *Ann Stomatol (Roma)*. 2012 Apr;3(2):75-81. Epub 2012 Aug 9. PMID: 23087790; PMCID: PMC3476496.

Objectives

The aim of this clinical trial was to compare clinical and biochemical healing outcomes following ultrasonic mechanical instrumentation versus ultrasonic mechanical instrumentation associated with topical subgingival application of amino acids and sodium hyaluronate gel.

Materials and method

Eleven systemically healthy subjects with moderate-severe chronic periodontitis, who had four sites with pocket probing depth and clinical attachment level greater than or equal to 5 mm were randomly assigned to two different types of treatment: two pockets were treated with ultrasonic debridement (Control Group) and two pockets with ultrasonic mechanical instrumentation associated with 0,5 ml of amino acids and sodium hyaluronate gel (Test Group). Probing depth, clinical attachment level, plaque index and bleeding on probing were recorded at baseline, 45 and 90 days. Levels of calprotectin and myeloperoxidase activity in gingival crevicular fluid were assessed at baseline and on day 7 and 45.

Results

Statistical significance was found between baseline and day 45 in relation to probing depth reduction and bleeding on probing between groups for both of the tested treatments. Significant reductions in $\mu\text{g/sample}$ of calprotectin and myeloperoxidase were found after 1-week and an increase at 45 days in both groups. There were no statistically significant differences between other variables evaluated in this study.

Conclusion

These data suggest that subgingival application of hyaluronic acid following ultrasonic mechanical instrumentation is beneficial for improving periodontal parameters.

Bürgers R, Witecy C, Hahnel S, Gosau M. The effect of various topical peri-implantitis antiseptics on *Staphylococcus epidermidis*, *Candida albicans*, and *Streptococcus sanguinis*. Arch Oral Biol. 2012;57(7):940-947. doi:10.1016/j.archoralbio.2012.01.015

Objective

Although peri-implantitis has presented an ever increasing problem in modern dentistry, satisfying therapeutic strategies or scientifically based treatment recommendations are still not available. The main object of the present study was to evaluate the antibacterial efficacy of six different topical antiseptics on three test microorganisms attached to titanium implant specimens.

Methodology

For biofilm formation, plane titan specimens were incubated either in *Candida albicans*, *Streptococcus sanguinis*, or *Staphylococcus epidermidis* for 2h. The specimens were then treated with different topical antiseptics for 60s (sodium hypochlorite 1.0%, hydrogen peroxide 3.0%, chlorhexidine gluconate 0.2%, citric acid 40.0%, Plax, or Listerine) and with sterile saline as control. Remaining vital fungi were quantified by means of a bioluminometric assay and the bacterial load and the viability of adhering *S. epidermidis* and *S. sanguinis* by live or dead cell labelling in combination with fluorescence microscopy.

Results

Sodium hypochlorite was effective against all three species, whereas hydrogen peroxide was solely effective against *C. albicans*. CHX and Listerine showed antimicrobial activity against *S. sanguinis* and *C. albicans* and citric acid and Plax against both tested bacteria.

Conclusion

None of the tested antimicrobial agents, except for sodium hypochlorite, showed a significant in vitro effect on all three test microbes. Considering the possible toxicity of sodium hypochlorite, none of the tested - and so far widely used - antiseptics showed any broad-spectrum antimicrobial effect and could therefore not be recommended for the topical disinfection and detoxification of infected implant surfaces.

Galván M, Gonzalez S, Cohen CL, et al. Periodontal effects of 0.25% sodium hypochlorite twice-weekly oral rinse. A pilot study. *J Periodontal Res.* 2014;49(6):696-702. doi:10.1111/jre.12151

Objective

The study aimed to evaluate the effect of 0.25% sodium hypochlorite twice-weekly oral rinse on plaque and gingivitis in patients with minimally treated periodontitis.

Methodology

The study included 30 patients with periodontitis, it lasted 3 mo, and it was performed as a randomized, controlled, single-blinded, clinical trial in parallel groups. Fifteen patients rinsed for 30 s with 15 mL of a fresh solution of 0.25% sodium hypochlorite (test) and 15 patients rinsed with 15 mL of water (control). Clorox(®) regular bleach was the source of the sodium hypochlorite. At baseline and at 2 wk, the study patients received professional subgingival irrigation for 5 min with either 0.25% sodium hypochlorite or water, but no subgingival or supragingival scaling. The presence or absence of supragingival plaque on facial and lingual surfaces was determined by visual inspection; each tooth was dried with air and mouth mirror rotation was used to provide light reflection to identify plaque on smooth surfaces and at the tooth line angles. Gingival bleeding within 30 s after probing to full pocket depth was assessed in six sites of each tooth. Adverse events were evaluated by questionnaire and visual examination.

Results

All 30 patients in the study completed the baseline and the 2 wk parts of the study and a subset of 12 participants completed the 3 mo part of the study. The sodium hypochlorite rinse group and the water rinse group, respectively, showed increases from baseline to 3 mo of 94% and 29% (3.2-fold difference) in plaque-free facial surfaces, of 195% and 30% (6.5-fold difference) in plaque-free lingual surfaces, and of 421% and 29% (14.5-fold difference) in number of teeth with no bleeding on probing. The differences in clinical improvement between the sodium hypochlorite rinse group and the water rinse group were statistically significant. No adverse events were identified in any of the study patients, except for minor complaints about the taste of bleach.

Conclusion

A twice-weekly oral rinse with 0.25% sodium hypochlorite produced marked **decreases in dental plaque level and bleeding on probing** and may constitute a promising new approach to the management of periodontal disease. Long-term controlled studies on the effectiveness of sodium hypochlorite oral rinse are needed and encouraged.

Gosau M, Hahnel S, Schwarz F, Gerlach T, Reichert TE, Bürgers R. Effect of six different peri-implantitis disinfection methods on in vivo human oral biofilm. Clin Oral Implants Res. 2010;21(8):866-872. doi:10.1111/j.1600-0501.2009.01908.x

Objective

The aim of this human in vivo pilot study was to evaluate the efficacy of six antimicrobial agents on the surface decontamination of an oral biofilm attached to titanium implants.

Methodology

For in vivo biofilm formation, we fixed titanium specimens to individual removable acrylic upper jaw splints (14 specimens in every splint), which were worn by four volunteers overnight for 12 h. The specimens were then treated with different antimicrobial agents for 1 min (Sodium hypochlorite, Hydrogen peroxide 3%, Chlorhexidyluconate 0.2%, Plax, Listerine, citric acid 40%). Afterwards, we quantified the total bacterial load and the viability of adhering bacteria by live or dead cell labelling in combination with fluorescence microscopy.

Results

The total bacterial load on the titanium surfaces was significantly higher after incubation in the control solution phosphate-buffered saline (PBS) than after disinfection in sodium hypochlorite, hydrogen peroxide, chlorhexidine, Plax, Listerine, and citric acid. Furthermore, a significantly lower ratio between dead and total adhering bacteria (bactericidal effect) was found after incubation in control PBS, Plax mouth rinse, and citric acid than after incubation in sodium hypochlorite, hydrogen peroxide, chlorhexidine, and Listerine.

Conclusion

All tested antiseptics seem to be able to reduce the total amount of microorganisms accumulating on titanium surfaces. Furthermore, **sodium hypochlorite**, hydrogen peroxide, chlorhexidine, and Listerine showed a **significant bactericidal effect against adhering bacteria**.

Gottardi W, Nagl M. N-chlorotaurine, a natural antiseptic with outstanding tolerability. *J Antimicrob Chemother.* 2010 Mar;65(3):399-409. doi: 10.1093/jac/dkp466. Epub 2010 Jan 6. PMID: 20053689.

Abstract

N-chlorotaurine, the N-chloro derivative of the amino acid taurine, is a long-lived oxidant produced by activated human granulocytes and monocytes. Supported by a high number of in vitro studies, it has mainly anti-inflammatory properties and seems to be involved in the termination of inflammation. The successful synthesis of the crystalline sodium salt (Cl-HN-CH₂-CH₂-SO₃Na, NCT) facilitated its development as an endogenous antiseptic. NCT can be stored long-term at low temperatures, and it has killing activity against bacteria, fungi, viruses and parasites. Transfer of the active chlorine to amino groups of molecules of both the pathogens and the human body (transhalogenation) enhances rather than decreases its activity, mainly because of the formation of monochloramine. Furthermore, surface chlorination after sublethal incubation times in NCT leads to a post-antibiotic effect and loss of virulence of pathogens, as demonstrated for bacteria and yeasts. Being a mild oxidant, NCT proved to be very well tolerated by human tissue in Phase I and II clinical studies. A 1% aqueous solution can be applied to the eye, skin ulcerations, outer ear canal, nasal and paranasal sinuses, oral cavity and urinary bladder, and can probably be used for inhalation. Therapeutic efficacy in Phase II studies has been shown in external otitis, purulently coated crural ulcerations and keratoconjunctivitis, so far. Based upon all presently available data, NCT seems to be an antiseptic with a very good relation between tolerability and activity. Recently, C-methylated derivatives of NCT have been invented, which are of interest because of improved stability at room temperature.

Gottardi W, Debabov D, Nagl M. N-chloramines, a promising class of well-tolerated topical anti-infectives. *Antimicrob Agents Chemother.* 2013 Mar;57(3):1107-14. doi: 10.1128/AAC.02132-12. Epub 2013 Jan 7. PMID: 23295936; PMCID: PMC3591902.

Abstract

Antibiotic resistance is a growing public health crisis. To address the development of bacterial resistance, the use of antibiotics has to be minimized for nonsystemic applications in humans, as well as in animals and plants. Possible substitutes with low potential for developing resistance are active chlorine compounds that have been in clinical use for over 180 years. These agents are characterized by pronounced differences in their chlorinating and/or oxidizing activity, with hypochlorous acid (HOCl) as the strongest and organic chloramines as the weakest members. Bacterial killing in clinical practice is often associated with unwanted side effects such as chlorine consumption, tissue irritation, and pain, increasing proportionally with the chlorinating/oxidizing potency. Since the chloramines are able to effectively kill pathogens (bacteria, fungi, viruses, protozoa), their application as anti-infectives is advisable, all the more so as they exhibit additional beneficial properties such as destruction of toxins, degradation of biofilms, and anticoagulative and anti-inflammatory activities. Within the ample field of chloramines, the stable N-chloro derivatives of β -aminosulfonic acids are most therapeutically advanced. Being available as sodium salts, they distinguish themselves by good solubility and absence of smell. Important representatives are N-chlorotaurine, a natural compound occurring in the human immune system, and novel mono- and dichloro derivatives of dimethyltaurine, which feature improved stability.

Kalkwarf KL, Tussing GJ, Davis MJ. Histologic evaluation of gingival curettage facilitated by sodium hypochlorite solution. J Periodontol. 1982;53(2):63-70. doi:10.1902/jop.1982.53.2.63

Abstract

Gingival curettage has long been used as a modality in periodontal therapy. Recent longitudinal studies have shown that diligent curettage of periodontal pockets may aid in maintaining the attachment levels around periodontally involved teeth. This study was designed to histologically evaluate the controlled use of concentrated sodium hypochlorite solution to facilitate gingival curettage therapy. The results indicate that the action of sodium hypochlorite solution may be adequately controlled to provide predictable chemolysis of the soft tissue wall of a periodontal pocket with **minimal effect upon the adjacent tissues**. The action of the solution appears to have no detrimental effect upon healing. Reduction of inflammation prior to the procedure and careful technique are important to assure predictability. **The use of gingival curettage augmented by sodium hypochlorite solution may aid in the management of patients undergoing periodontal maintenance.**

Mohammadi Z. Sodium hypochlorite in endodontics: an update review. *Int Dent J.* 2008;58(6):329-341. doi:10.1111/j.1875-595x.2008.tb00354.x

Abstract

The major objective in root canal treatment is to disinfect the entire root canal system. This requires that the pulpal contents be eliminated as sources of infection. This goal may be accomplished using mechanical instrumentation and chemical irrigation, in conjunction with medication of the root canal between treatment sessions. Microorganisms and their by-products are considered to be the major cause of pulpal and periradicular pathosis. In order to reduce or eliminate bacteria and pulpal tissue remnants, various irrigation solutions have been suggested to be used during treatment. **Sodium hypochlorite, an excellent non-specific proteolytic and antimicrobial agent, is the most common irrigation solution** used during root canal therapy. The purpose of this paper was to review different aspects of sodium hypochlorite use in endodontics.

Relevant Studies on hyaDENT BG

Asparuhova M, Chappuis V, Stähli A, Buser D, Sculean A, 'Role of hyaluronan in regulating self-renewal and osteogenic differentiation of mesenchymal stromal cells and pre-osteoblasts, Clin Oral Investig. 2020 Mar 31. doi: 10.1007/s00784-020-03259-8.

Objectives

The aim of the study was to investigate the impact of two hyaluronan (HA) formulations on the osteogenic potential of osteoblast precursors.

Methodology

Proliferation rates of HA-treated mesenchymal stromal ST2 and pre-osteoblastic MC3T3-E1 cells were determined by 5-bromo-20-deoxyuridine (BrdU) assay. Expression of genes encoding osteogenic differentiation markers, critical growth, and stemness factors as well as activation of downstream signalling pathways in the HA-treated cells were analysed by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and immunoblot techniques.

Results

The investigated HAs strongly stimulated the growth of the osteoprogenitor lines and **enhanced the expression of genes encoding bone matrix proteins**. However, expression of late osteogenic differentiation markers was significantly inhibited, accompanied by decreased bone morphogenetic protein (BMP) signalling. The expression of genes encoding transforming growth factor- β 1 (TGF- β 1) and fibroblast growth factor-1 (FGF-1) as well as the phosphorylation of the downstream signalling molecules Smad2 and Erk1/2 were enhanced upon HA treatment. We observed significant upregulation of the transcription factor Sox2 and its direct transcription targets and critical stemness genes, Yap1 and Bmi1, in HA-treated cells. Moreover, prominent targets of the canonical Wnt signalling pathway showed reduced expression, whereas inhibitors of the pathway were considerably upregulated. We detected decrease of active β -catenin levels in HA-treated cells due to β -catenin being phosphorylated and, thus, targeted for degradation.

Conclusions

HA strongly induces the **growth of osteoprogenitors and maintains their stemness**, thus potentially regulating the balance between self-renewal and differentiation during bone regeneration following reconstructive oral surgeries.

Clinical relevance

Addition of HA to deficient bone or bony defects during implant or reconstructive periodontal surgeries may be a viable approach for **expanding adult stem cells without losing their replicative and differentiation capabilities**.

Asparuhova M, Kiryak D, Eliezer M, Mihov D, Sculean A. 'Activity of two hyaluronan preparations on primary human oral fibroblasts'. J Periodontal Res 2018 Sep 27. Epub 2018 Sep 27

Objective

The potential benefit of using hyaluronan (HA) in reconstructive periodontal surgery is still a matter of debate. The aim of the present study was to evaluate the effects of two HA formulations on human oral fibroblasts involved in soft tissue wound healing / regeneration.

Methodology

Metabolic, proliferative, and migratory abilities of primary human palatal and gingival fibroblasts were examined upon HA treatment. To uncover the mechanisms whereby HA influences cellular behaviour, wound healing-related gene expression and activation of signalling kinases were analysed by qRT-PCR and Results: The investigated HA formulations maintained the viability of oral fibroblasts, and increased their proliferative and migratory abilities. They enhanced expression of genes encoding type III collagen and transforming growth factor- β 3, characteristic of scarless wound healing. The HAs upregulated the expression of genes encoding pro-proliferative, pro-migratory, and pro-inflammatory factors, with only a moderate effect on the latter in gingival fibroblasts. In palatal but not gingival fibroblasts, an indirect effect of HA on the expression of matrix metalloproteinases 2 and 3 was detected, potentially exerted through induction of pro-inflammatory cytokines. Finally, our data pointed on Akt, Erk1/2 and p38 as the signalling molecules whereby the HAs exert their effects on oral fibroblasts.

Conclusion

Both investigated 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. Our data further suggest that in gingival tissues, the HAs are not likely to impair the healing process by prolonging **inflammation** or causing excessive MMP expression at the repair site.

Eliezer M, Imber JC, Sculean A, Pandis N, Teich S, 'Hyaluronic acid as adjunctive to non-surgical and surgical periodontal therapy: a systematic review and meta-analysis', Clin O Inv 2019; doi: s00784-019-03012-w

Objectives

To evaluate the potential added benefit of the topical application of hyaluronic acid (HA) on the clinical outcomes following non-surgical or surgical periodontal therapy.

Materials and method

A systematic search was performed in Medline, Embase, Cochrane, Web of Science, Scopus and Grey literature databases. The literature search was performed according to PRISMA guidelines. The Cochrane risk of bias tool was used in order to assess the methodology of the included trials. Weighted mean differences (WMDs) and 95% confidence intervals (CIs) between the treatment and controls were estimated using the random-effect model for amount of bleeding on probing (BOP), probing depth (PD) reduction and clinical attachment level (CAL) gain. In order to minimize the bias and to perform meta-analysis, only randomized clinical studies (RCTs) were selected.

Results

Thirteen RCTs were included: 11 on non-surgical periodontal treatment and two on surgical periodontal treatment. Overall analysis of PD reduction, CAL gain and BOP reduction in non-surgical therapy with adjunctive HA presented WMD of - 0.36 mm (95% CI - 0.54 to - 0.19 mm; $p < 0.0001$), 0.73 mm (95%CI 0.28 to 1.17 mm; $p < 0.0001$) and - 15% (95% CI - 22 to - 8%; $p < 0.001$) respectively, favouring the application of HA. The overall analysis on PD and CAL gain in surgical therapy with adjunctive HA presented WMD of - 0.89 mm (95% CI - 1.42 to - 0.36 mm; $p < 0.0001$) for PD reduction and 0.85 mm (95% CI 0.08 to 1.62 mm; $p < 0.0001$) for CAL gain after 6–24 months favouring the treatment with HA. However, comparison presented considerable heterogeneity between the non-surgical studies and a high risk of bias in general.

Conclusion

Within their limits, the present data indicate that the topical application of HA may lead to additional clinical benefits when used as an adjunctive to non-surgical and surgical periodontal therapy. However, due to the high risk of bias and heterogeneity, there is a need for further well-designed RCTs to evaluate this material in various clinical scenarios.

Clinical relevance

The adjunctive use of HA may improve the clinical outcomes when used in conjunction with non-surgical and surgical periodontal therapy.

Fujioka-Kobayashi M, Müller H, Mueller A, Lussi A, Sculean A, Schmidlin PR, Miron RJ 'In vitro effects of hyaluronic acid on human periodontal ligament cells' BMC Oral Health (2017) 17:44 DOI 10.1186/s12903-017-0341-1

Background

Hyaluronic acid (HA) has been reported to have a positive effect on periodontal wound healing following nonsurgical and surgical therapy. However, to date, a few basic in vitro studies have been reported to investigating the potential of HA on human periodontal ligament (PDL) cell regeneration. Therefore, the aim of this study was to investigate the effect of HA on PDL cell compatibility, proliferation, and differentiation in vitro.

Methodology

Either non-cross-linked (HA_ncl) or cross-linked (HA_cl) HA was investigated. Human PDL cells were seeded in 7 conditions as follows (1) Control tissue culture plastic (TCP) (2) dilution of HA_ncl (1:100), (3) dilution of HA_ncl (1:10), 4) HA_ncl directly coated onto TCP, (5) dilution of HA_cl (1:100), 6) dilution of HA_cl (1:10) and (7) HA_cl directly coated onto TCP. Samples were then investigated for cell viability using a live/dead assay, an inflammatory reaction using real-time PCR and ELISA for MMP2, IL-1 and cell proliferation via an MTS assay. Furthermore, the osteogenic potential of PDL cells was assessed by alkaline phosphatase(ALP) activity, collagen1(COL1) and osteocalcin(OCN) immunostaining, alizarin red staining, and real-time PCR for genes encoding Runx2, COL1, ALP, and OCN.

Results

Both HA_ncl and HA_cl showed **high PDL cell viability** (greater than 90%) irrespective of the culturing conditions. Furthermore, no significant difference in both mRNA and protein levels of proinflammatory cytokines, including MMP2 and IL-1 expression was observed. 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 decreased ALP staining, COL1 immunostaining and down-regulated early osteogenic differentiation, including Runx2, COL1, and OCN mRNA levels when compared to control samples. When osteogenic differentiation medium (ODM) was added, interestingly, the expression of early osteogenic markers increased by demonstrating higher levels of COL1 and ALP expression; especially in HA 1:10 diluted condition. Late stage osteogenic markers remained inhibited.

Conclusions

Both non-cross-linked and cross-linked **HA maintained high PDL cell viability, increased proliferation, and early osteogenic differentiation**. However, HA was consistently associated with a significant decrease in late osteogenic differentiation of primary human PDL cells. Future in vitro and animal research is necessary to further characterize the effect of HA on periodontal regeneration.

Mueller A, Fujioka-Kobayashi M, Mueller HD, Lussi A, Sculean A, Schmidlin PR, Miron RJ. 'Effect of hyaluronic acid on morphological changes to dentin surfaces and subsequent effect on periodontal ligament cell survival, attachment, and spreading' *Clinical Oral Investigations* 2016 May .DOI 10.1007/s00784-016-1856-6

Objectives

Hyaluronic acid (HA) is a natural constituent of connective tissues and plays an important role in their development, maintenance, and regeneration. Recently, **HA has been shown to improve wound healing**. However, no basic in vitro study to date has investigated its mode of action. Therefore, the purpose of this study was to examine morphological changes of dentin surfaces following HA coating and thereafter investigate the influence of periodontal ligament (PDL) cell survival, attachment, and spreading to dentin discs.

Methodology

HA was coated onto dentin discs utilizing either non-cross-linked (HA) or cross-linked (HA cl) delivery systems. Morphological changes to dentin discs were then assessed using scanning electron microscopy (SEM). Thereafter, human PDL cells were seeded under three in vitro conditions including (1) dilution of HA (1:100), (2) dilution of HA (1:10), and (3) HA coated directly to dentin discs. Samples were then investigated for PDL cell survival, attachment, and spreading using a live/dead assay, cell adhesion assay, and SEM imaging, respectively.

Results

While control dentin discs demonstrated smooth surfaces both at low and high magnification, the coating of HA altered surface texture of dentin discs by increasing surface roughness. HA cl further revealed greater surface texture/roughness likely due to the cross-linking carrier system. Thereafter, PDL cells were seeded on control and HA coated dentin discs and demonstrated a near 100 % survival rate for all samples demonstrating high biocompatibility of HA at dilutions of both 1:100 and 1:10. Interestingly, non-cross-linked HA significantly increased cell numbers at 8 h, whereas cross-linked HA improved cell spreading as qualitatively assessed by SEM.

Conclusions

The results from the present study demonstrate that both carrier systems for **HA were extremely biocompatible and demonstrated either improved cell numbers or cell spreading onto dentin discs**. Future in vitro and animal research is necessary to further characterize the optimal delivery system of HA for improved clinical use.

Clinical relevance

HA is a highly **biocompatible material that may improve PDL cell attachment** or spreading on dentin.

Pilloni A, Schmdlin PR, Sahrman P, Sculean A, Rojas MA. 'Effectiveness of adjunctive hyaluronic acid application in coronally advanced flap in Miller class I single gingival recession sites: a randomized controlled clinical trial, Clinical Oral Investigations, 2018 <https://doi.org/10.1007/s00784-018-2537-4>

Objectives

The aim of this randomized controlled clinical trial was to evaluate the possible advantages of adjunctive hyaluronic acid (HA) application in the coronally advanced flap (CAF) procedure in single Miller class I/recession type 1 (RT1) gingival recession treatment.

Methodology

Thirty patients with one recession were enrolled; 15 were randomly assigned CAF + HA and 15 to CAF alone. The recession reduction (RecRed), clinical attachment level gain (CAL-gain), changes in probing pocket depth (PPD) and in the width of keratinized tissue (KT), complete root coverage (CRC), and mean root coverage (MRC) were calculated after 18 months. Post-operative morbidity (pain intensity, discomfort, and swelling) was recorded 7 days after treatment using visual analogue scale (VAS).

Results

After 18 months, RecRed was statistically significantly higher in the test group (2.7 mm [1.0]) than in the control group (1.9 mm [1.0]; $p = 0.007$). PPD were found to be slightly but statistically significantly increased in both groups. No statistically significant difference was found for KT gain between treatments. CRC was 80% for test and 33.3% for control sites ($p < 0.05$). A MRC of $93.8 \pm 13.0\%$ for test and $73.1 \pm 20.8\%$ for control sites was calculated ($p < 0.05$). The test group reported lower swelling and discomfort values 7-days post-surgery ($p < 0.05$). Statistically significant difference was not found for pain intensity.

Conclusions

The adjunctive use of **HA was effective in obtaining CRC** for single Miller class I/RT1 gingival recession sites.

Clinical relevance

Adjunctive application of **HA in the coronally advanced flap procedure may improve the reduction of the recessions and increase the probability of CRC** in Miller class I recessions.

Shirakata Y, Imafuji T, Nakamura T, Shinohara Y, Iwata M, Setoguchi F, Noguchi K, Sculean A. Cross-linked hyaluronic acid gel with or without a collagen matrix in the treatment of class III furcation defects: A histologic and histomorphometric study in dogs. *J Clin Periodontol*. 2022 Oct;49(10):1079-1089. doi: 10.1111/jcpe.13694. Epub 2022 Jul 21. PMID: 35817414; PMCID: PMC9796036.

Objectives

To histologically evaluate the effects of cross-linked hyaluronic acid (xHyA) with or without a collagen matrix (CM) on periodontal wound healing/regeneration in class III furcation defects in dogs.

Material and methods

Class III furcation defects were surgically created in the mandibular premolars in six beagle dogs. The defects were randomly treated as follows: open flap debridement (OFD) + CM (CM), OFD + xHyA (xHyA), OFD + xHyA + CM (xHyA/CM) and OFD alone (OFD). At 10 weeks, the animals were euthanized for histological evaluation.

Results

The newly formed bone areas in the xHyA ($4.04 \pm 1.51 \text{ mm}^2$) and xHyA/CM ($4.32 \pm 1.14 \text{ mm}^2$) groups were larger than those in the OFD ($3.25 \pm 0.81 \text{ mm}^2$) and CM ($3.31 \pm 2.26 \text{ mm}^2$) groups. The xHyA ($6.25 \pm 1.45 \text{ mm}$) and xHyA/CM ($6.40 \pm 1.35 \text{ mm}$) groups yielded statistically significantly ($p < .05$) greater formation of new connective tissue attachment (i.e., new cementum, with inserting connective tissue fibres) compared with the OFD ($1.47 \pm 0.85 \text{ mm}$) group. No significant differences were observed in any of the histomorphometric parameters between the xHyA and xHyA/CM groups. Complete furcation closure was not observed in any of the four treatment modalities.

Conclusion

Within their limits, the present results suggest that the use of xHyA with or without CM positively influences periodontal wound healing in surgically created, acute-type class III furcation defects.

Shirakata Y, Nakamura T, Kawakami Y, et al. Healing of buccal gingival recessions following treatment with coronally advanced flap alone or combined with a cross-linked hyaluronic acid-gel. An experimental study in dogs. Journal of Clinical Periodontology. 2021 Jan. DOI: 10.1111/jcpe.13433.

Objectives

To clinically and histologically evaluate in dogs the healing of gingival recessions treated with coronally advanced flap (CAF) with or without cross-linked hyaluronic acid (HA).

Material and methods

Gingival recession defects were surgically created on the vestibular side of both maxillary canines in 8 dogs. After 8 weeks of plaque accumulation, the 16 chronic defects were randomly treated with either CAF alone or CAF and HA-gel (CAF/HA). Clinical and histological outcomes were evaluated at 10 weeks post surgically.

Results

Compared to baseline, the clinical measurements at 10 weeks revealed a statistically significant decrease in gingival recession for both CAF ($p < 0.01$) and CAF/HA ($p < 0.001$) groups. Statistically significant differences were found in clinical attachment level ($p < 0.05$) and width of gingival recession ($p < 0.01$) favoring the CAF/HA group. Bone formation was statistically significantly greater in the CAF/HA group than in the CAF group ($1.84 \pm 1.16\text{mm}$ vs., $0.72 \pm 0.62\text{mm}$ respectively, $P < 0.05$). Formation of cementum and connective tissue attachment were statistically significantly higher in the CAF/HA group compared with the CAF group (i.e. $4.31 \pm 1.78\text{mm}$ versus $2.40 \pm 1.35\text{mm}$ and $1.69 \pm 0.98\text{mm}$ versus $0.74 \pm 0.68\text{mm}$, respectively ($P < 0.05$)).

Conclusion

The present data have for the first time provided histologic **evidence for periodontal regeneration of gingival recession** defects following treatment with CAF and HA.

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[Healing of buccal gingival recessions following treatment with coronally advanced flap alone or combined with a cross-linked hyaluronic acid gel. An experimental study in dogs \(nih.gov\)](#)

Shirakata Y, Imafuji T, Nakamura T, Kawakami Y, Shinohara Y, Noguchi K, Pilloni A, Sculean A. Periodontal wound healing/regeneration of two-wall intrabony defects following reconstructive surgery with cross-linked hyaluronic acid-gel with or without a collagen matrix: a preclinical study in dogs. *Quintessence Int.* 2021;0(0):308-316. doi: 10.3290/j.qi.b937003. PMID: 33533237.

Objectives

Histologically evaluate the effects of cross-linked HA alone or combined with a collagen matrix (CM = Fibro Gide) on the periodontal wound healing/regeneration in intrabony defects.

Material and methods

Two-wall intrabony defects (5 mm wide, 5 mm deep) were surgically created at the distal and mesial aspects of mandibular premolars in six beagle dogs. The 24 defects were randomly treated as follows: open flap debridement (OFD) + HA, OFD +CM, OFD + HA+ CM (HA/CM) and OFD alone (control). At 2 months, the animals were euthanized for histological evaluation.

Results

The HA (2.43 ± 1.25 mm) and HA/CM (2.60 ± 0.99 mm) groups yielded statistically significantly ($P < 0.05$) greater formation of new attachment (i.e., linear length of NC adjacent to newly formed bone, with inserting collagen fibers) compared with OFD (0.55 ± 0.99 mm) group. Among the 4 treatment groups, the HA/CM group demonstrated the highest amount of regenerated tissues, although no statistically significant differences in any of the histometric parameters were observed between the HA and HA/CM groups.

Within their limits, it can be concluded that cross-linked HA alone or combined with CM promotes periodontal wound healing/ regeneration in two-wall intrabony defects in dogs.

Conclusion

The present data have for the **first time provided histologic evidence for periodontal regeneration of gingival recession defects following treatment with CAF and HA.**

Relevant Studies on Hyaluronic Acid

Jimbo R, Singer J, Tovar N, Marin C, Neiva R, Bonfante EA, Janal MN, Contamin H, Coelho PG, "Regeneration of the cementum and periodontal ligament using local BDNF delivery in class II furcation defects.", J Biomed Mater Res B Appl Biomater. 2018 May;106(4):1611-1617. doi: 10.1002/jbm.b.33977. Epub 2017 Aug 21

Objectives

Periodontal furcation defects are usually addressed by the placement of a physical barrier which may limit the regenerative potential of periodontal wounds.

Methodology

This study morphometrically quantified the regenerative effect of brain-derived neurotrophic factor (BDNF) in furcation defects in a non-human primate model. Grade II furcation defects (with and without induced inflammation prior to surgery) were created on the first and second molars of eight non-human primates. Defects were treated with open flap debridement and subsequently filled with either: Group A; BDNF (500 $\mu\text{g mL}^{-1}$) in high-molecular weight-hyaluronic acid (HMW-HA), Group B; BDNF (50 $\mu\text{g mL}^{-1}$) in HMW-HA, Group C; HMW-HA acid only, Group D; unfilled defect, or Group E; BDNF (500 $\mu\text{g mL}^{-1}$) in saline. Periodontal wound healing was observed every 2 weeks by computed-tomography. At 11 weeks all animals were sacrificed and maxillary and mandibular block biopsies were referred for nondecalcified histology.

Results

Linear measurements of new cementum (cellular and acellular) and periodontal ligament (PDL) formation were performed. Computerized-tomography reconstruction and software quantification demonstrated successful bone fill for all groups. However, histometric assessment demonstrated significantly higher level of total periodontal regeneration for the 500 $\mu\text{g mL}^{-1}$ BDNF HMW-HA relative to all other groups.

Conclusion

No significant differences in cementogenesis were observed among groups. Significantly higher acellular cementum formation was observed for sites where inflammation was not induced prior to surgical procedures. While all groups experienced similar bone fill and cementogenesis, the 500 $\mu\text{g mL}^{-1}$ BDNF **HMW-HA appeared to most effectively repair PDL** (minimum increase of ~22% relative to all groups; over 200% relative to unfilled defects).

King, S.R., Hickerson, W.L. and Proctor, K.G. (1991) Beneficial Actions of Exogenous Hyaluronic Acid on Wound Healing. *Surgery*, 109, 76-86.

Objective

To determine the effect of exogenous hyaluronic acid (HA) on healing of experimental wounds, responses in the hamster cheek pouch were measured after a hole was cut through the tissue with a biopsy punch.

Methodology

Fluorescence-labeled dextran was administered intravenously as a macromolecular tracer and the microcirculation was observed in vivo with a fluorescence microscope connected to a high-resolution television system. In one group a gelatin sponge soaked in 1.5 ml 16 mg/dl HA in water was applied topically at the time of injury and on postinjury days 1, 3, 5, and 7. The control group received the sponge soaked in the aqueous vehicle. Every 2 days after injury, the microcirculation was observed or histologic specimens were harvested. Wound size decreased almost twice as fast with HA compared with its vehicle (p less than 0.05). Healing was defined as time for total wound closure with at least one microvessel bridging the site of injury and required 16 or more days with vehicle but averaged less than 9 days with HA.

Results

Early during healing the repair site was surrounded by widespread extravasation of the fluorescent tracer, an index of inflammation; this area was reduced by two thirds 2 to 4 days after injury with HA compared with its vehicle (p less than 0.05). The density of perfused microvessels was twofold higher with HA 2 to 4 days after injury (p less than 0.05). However, microvessel density was similar in both groups by 6 days after injury and remained similar for at least 45 days after injury, which suggests that HA evoked no unusual angiogenic response. Histologic examination of fixed, stained specimens showed increases in intravascular leukocytes after injury and treatment-related differences in the distribution of intravascular leukocytes in 20 to 40 microns and 40 to 80 microns diameter microvessels 1 to 2 days after injury. Otherwise, leukocyte infiltration during healing was similar in both groups.

Conclusion

The mechanism for the beneficial action of HA on healing is unknown. However, several in vitro studies suggest that **HA is part of a feedback loop that promotes cell proliferation and migration in actively growing tissues**. Alternatively, the role of HA in water homeostasis could favor tissue hydration, which has a well-known beneficial **effect on healing**.

Pirnazar P, Wolinsky L, Nachnani S, Haake S, Pilloni A, Bernard GW. 'Bacteriostatic effects of hyaluronic acid.' J Periodontol 1999;70:370-4.

Background

This investigation is one of a series of projects seeking to ascertain whether hyaluronic acid (HA) is therapeutically effective in tissue regeneration procedures. The rationale for these investigations is to test the hypothesis that HA can serve as a bioabsorbable carrier for other substrates as well as itself actively promote the regeneration of tissue.

Methods

In this paper, we report on the bacteriostatic and bactericidal properties of 3 molecular weight formulations of recombinant HA (low, 141 kD; medium, 757 kD; and high, 1,300 kD) on selected oral and non-oral microorganisms in the planktonic phase. Three concentrations of each HA formulation were screened, 0.5, 1.0, and 2.0 mg/ml, using a standard broth culture assay.

Results

Recombinant HA exerted varied bacteriostatic effects on all the bacterial strains tested depending on its molecular weight (MW) and concentration. The high concentrations of the medium MW HA had the greatest bacteriostatic effect, particularly on the *Actinobacillus actinomycetemcomitans*, *Prevotella oris*, *Staphylococcus aureus*, and *Propionibacterium acnes* strains. The 1.0 mg/ml concentration of high MW HA had the greatest overall bacteriostatic effect, inhibiting the growth of all 6 bacterial strains tested. Among the bacterial strains studied, HA was found to have no bactericidal effects, regardless of concentration or molecular weight.

Conclusions

The results of this study suggest that HA in the MW range of 1,300 kD may prove beneficial in **minimizing bacterial contamination of surgical wounds** when used in guided tissue regeneration surgery.

Sasaki T, Watanabe C, Stimulation of Osteoinduction in Bone Wound Healing by High-Molecular Hyaluronic Acid. Bone. Vol. 16. No.1 January 1995:9-15

Objective

To study the osteoinductive action of hyaluronic acid (HA), we examined the effects of applying an elastoviscous high molecular HA preparation on bone wound healing after bone marrow ablation.

Methodology

The middiaphyses of cortical bones from rat femurs were perforated with a round bar, and excavated marrow cavities were filled immediately with high-molecular HA. Bone marrow ablation without HA was used to prepare controls. On post-ablation days 1, 2, 4, 7, and 14, animals were perfusion-fixed with an aldehyde mixture, and dissected femurs were examined by means of light, transmission-, and scanning-electron microscopy. In controls, the wounded marrow cavities were first filled with blood and fibrin clots (days 1 and 2), then with granulated tissues containing macrophages, neutrophils, and fibroblastic cells (day 4).

Results

New bone formation by differentiated osteoblasts was observed at 1-week post-ablation; at 2 weeks, the perforated cortical bones and marrow cavities were filled mostly with newly formed trabecular bone. In bones to which HA had been applied, new bone formation already had been induced by day 4 on both the peri- and endosteal surfaces of the existing cortical bones. At 1-week post-ablation, marrow cavities were completely filled with newly formed trabecular bones, in which active bone remodelling by osteoblasts and osteoclasts had occurred. Granulated tissues were replaced rapidly by normal marrow cells.

Conclusion

These results suggest that **high-molecular HA is capable of accelerating new bone formation** through mesenchymal cell differentiation in bone wounds.

Yıldırım S, Özener HÖ, Doğan B, Kuru B. Effect of topically applied hyaluronic acid on pain and palatal epithelial wound healing: An examiner-masked, randomized, controlled clinical trial. J Periodontol. 2018;89(1):36-45. doi:10.1902/jop.2017.170105

Objectives

This study aims to evaluate the effects of two different concentrations of topical hyaluronic acid (HA) on postoperative patient discomfort and wound healing of palatal donor sites after free gingival graft (FGG) surgery.

Methodology

Thirty-six patients requiring FGG were randomly assigned into three groups in an examiner-masked, randomized, controlled clinical trial. After harvesting palatal grafts, 0.2% and 0.8% HA gels were used in test groups 1 and 2, respectively. Gels were applied on donor sites and protected with periodontal dressing in the test groups, whereas the wound was covered only with periodontal dressing in the control group. On days 3, 7, 14, and 21, pain and burning sensation were recorded using a visual analog scale (VAS) as well as other parameters such as complete epithelization (CE) and color match on days 3, 7, 14, 21, and 42.

Results

Test groups experienced less pain than the control group on days 3 and 7 ($P < 0.001$ and $P < 0.001$, respectively). Mean VAS score for burning sensation was higher in the control group on day 3 compared with test groups 1 and 2 ($P = 0.03$ and $P = 0.02$, respectively). CE in all patients was achieved on day 21 in both test groups, whereas it was achieved on day 42 in the control group. The test groups showed higher color match scores than the control group on days 21 ($P < 0.001$ and $P < 0.001$, respectively) and 42 ($P = 0.004$ and $P = 0.002$, respectively).

Conclusion

Topical application of HA exhibits **positive impact on postoperative pain and burning sensation, and accelerates palatal wound healing** in terms of epithelization and color match.

Aya KL, Stern R. Hyaluronan in wound healing: rediscovering a major player. *Wound Repair Regen.* 2014;22(5):579-593. doi:10.1111/wrr.12214

Abstract

Wound healing involves a series of carefully modulated steps, from initial injury and blood clot to the final reconstituted tissue or scar. A dynamic reciprocity exists throughout between the wound, blood elements, extracellular matrix, and cells that participate in healing. Multiple cytokines and signal transduction pathways regulate these reactions. A major component throughout most of the process is hyaluronan, a straight-chain carbohydrate extracellular matrix polymer. Hyaluronan occurs in multiple forms, chain length being the only distinguishing characteristic between them. Levels of hyaluronan in its **high-molecular-weight form are prominent in the earliest stages of wound repair**. Progressively more fragmented forms occur in a manner not previously appreciated. We outline here steps in the wound healing cascade in which hyaluronan participates, as well as providing a review of its metabolism. Although described by necessity in a series of quantum steps, the healing process is constituted by a smooth continuum of overlapping reactions. The prevalence of hyaluronan in the wound (initially termed "hexosamine-containing mucopolysaccharide"), particularly in its early stages, was pointed out over half a century ago by the Harvard surgeon J. Engelbert Dunphy. It appears we are now returning to where we started.

Fawzi KM et al. 'Local application of hyaluronan gel in conjunction with periodontal surgery: a randomized controlled trial.' *Clinical Oral Investigations*, 2012; 16, 1229–1236.

Objectives

Hyaluronic acid application has been proven to be beneficial in a number of medical disciplines. The aim of the current study was to clinically evaluate the effect of local application of hyaluronan gel in conjunction with periodontal surgery.

Methodology

Fourteen patients with chronic periodontitis having four interproximal intrabony defects (≥ 3 mm) with probing depth values > 5 mm were included in this split-mouth study. Following initial nonsurgical periodontal therapy and re-evaluation, defects were randomly assigned to be treated with modified Widman flap (MWF) surgery in conjunction with either 0.8% hyaluronan gel (test) or placebo gel (control) application. Clinical attachment level (CAL), probing depth (PD), gingival recession (GR), plaque index (PI), and bleeding on probing (BOP) values were taken at baseline and 3 and 6 months. Differences between test and control sites were evaluated using a Wilcoxon signed-rank and a McNemar test. A Friedman and a Cochran test were used to test equal ranks over time. Statistically significant differences were noted for CAL and GR ($P < 0.05$) in favor of the test sites.

Results

No significant differences were found regarding PD, BOP, or PI values ($P > 0.05$).

Conclusion

Hyaluronan gel application in conjunction with periodontal surgery appears to result in **significant improvement of CAL and in a reduction in GR**. Hyaluronan gel application appears to improve the clinical outcome of MWF surgery.