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SCIENTIFIC EVIDENCE OF HYALURONIC ACID IN DENTAL REGENERATION

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Overview of Publications

	NON-SURGICAL	SURGICAL			
	Periodontal & Peri-implant infections	Intra bony defects	Furcation	Gingival recession	GBR
Husseini 2023 new					xHyA
Eliezer 2022 new					xHyA
Nobis 2022 new		xHyA	xHyA		
Diehl 2022 new	xHyA				
Shirakata 2022 new			xHyA		
Cervino 2021					xHyA
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Shirakata 2021				xHyA	
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Eliezer 2019		xHyA		xHyA	xHyA
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Pilloni 2018				xHyA	
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Mendes 2008					HyA
Muzaffer 2006					HyA
Pirnazar 1999		HyA	HyA	HyA	HyA
Sasaki 1995		HyA	HyA		HyA
King 1991	HyA	HyA	HyA	HyA	HyA

Note:

HyA = studies with generic hyaluronic acid

xHyA = studies about the cross-linked hyaluronic acid marketed by Regedent

Non-surgical Periodontology

Periodontitis

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 peridodontal 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

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 preformed 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.

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-ß3, 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.

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 he**aling 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.

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**.

Surgical Periodontology

Intra-bony defects

Nobis, B.; Ostermann, T.; Weiler, J.; Dittmar, T.; Friedmann, A. Impact of Cross-Linked Hyaluronic Acid on Osteogenic Differentiation of SAOS-2 Cells in an Air-Lift Model. Materials 2022, 15, 6528. https:// doi.org/10.3390/ma15196528

Background
The aim of this study was to investigate the impact of cross-linked hyaluronic acid on osteoblast-like cells seeded on top of two collagen substrates, native porcine pericardium membrane (substrate A) and ribose cross-linked collagen membranes (substrate B), in an air-lift model.

Procedure
Substrates A or B, saturated with three hyaluronic acid concentrations, served as membranes for SAOS-2 cells seeded on top. Cultivation followed for 7 and 14 days in the air-lift model. Controls used the same substrates without hyaluronic pre-treatment. Cells were harvested, and four (Runx2, BGLAP, IBSP, Cx43) different osteogenic differentiation markers were assessed by qPCR. Triplicated experiment outcomes were statistically analyzed (ANOVA, t-test; SPSS).

Outcome
Supplementary histologic analysis confirmed the cells' vitality. After seven days, only few markers were overexpressed on both substrates. After 14 days, targeted genes were highly expressed on substrate A. The same substrate treated with 1:100 diluted xHyA disclosed statistically significant different expression level vs. substrate B ($p = 0.032$). Time ($p = 0.0001$), experimental condition as a function of time ($p = 0.022$), and substrate ($p = 0.028$) were statistically significant factors. Histological imaging demonstrated vitality and visualized nuclei.

Conclusion
We conclude that the impact of hyaluronic acid resulted in a higher expression profile of SAOS-2 cells on substrate A compared to substrate B in an air-lift culture after two weeks.

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https://www.mdpi.com/1996-1944/15/19/6528/pdf

Božić D, Ćatović I, Badovinac A, Musić L, Par M, Sculean A. Treatment of Intrabony Defects with a Combination of Hyaluronic Acid and Deproteinized Porcine Bone Mineral. Materials (Basel). 2021 Nov 11;14(22):6795. doi: 10.3390/ma14226795. PMID: 34832196; PMCID: PMC8624958.

Background
This study evaluates the clinical outcomes of a novel approach in treating deep intrabony defects utilizing papilla preservation techniques with a combination of hyaluronic acid (xHyA) and deproteinized porcine bone mineral.

Clinical Procedure
23 patients with 27 intrabony defects were treated with a combination of HA and deproteinized porcine bone mineral. Clinical attachment level (CAL), pocket probing depth (PPD), gingival recession (REC) were recorded at baseline and 6 months after the surgery.

Outcomes
At 6 months, there was a significant CAL gain of 3.65 ± 1.67 mm ($p < 0.001$) with a PPD reduction of 4.54 ± 1.65 mm ($p < 0.001$), which was associated with an increase in gingival recession (0.89 ± 0.59 mm, $p < 0.001$). The percentage of pocket resolution based on a PPD ≤4 mm was 92.6% and the failure rate based on a PPD of 5 mm was 7.4%.

Conclusion
The present findings indicate that **applying a combined HA and xenograft approach in deep intrabony defects provides clinically relevant CAL gains and PPD reductions compared to baseline values and is a valid new approach in treating intrabony defects**.

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Treatment of Intrabony Defects with a Combination of Hyaluronic Acid and Deproteinized Porcine Bone Mineral (nih.gov)

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 ± 1.51 mm2) and xHyA/CM (4.32 ± 1.14 mm2) groups were larger than those in the OFD (3.25 ± 0.81 mm2) and CM (3.31 ± 2.26 mm2) groups. The xHyA (6.25 ± 1.45 mm) and xHyA/CM (6.40 ± 1.35 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 ± 0.85 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, 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**.

Pilloni A, Rojas MA, Marini L, Russo P, Shirakata Y, Sculean A, Iacono R. Healing of intrabony defects following regenerative surgery by means of single-flap approach in conjunction with either hyaluronic acid or an enamel matrix derivative: a 24-month randomized controlled clinical trial. Clin Oral Investig. 2021 Aug;25(8):5095-5107. doi: 10.1007/s00784-021-03822-x. Epub 2021 Feb 10. PMID: 33565017; PMCID: PMC8342388.

Objectives
Enamel matrix derivative (EMD) in combination with flap designs aiming to maximally preserve the interdental soft tissues, is still considered the gold standard in the regenerative treatment of periodontal intrabony defects. However, increasing evidence from preclinical and clinical studies indicates that hyaluronic acid (HA) possesses a number of positive biologic effects on periodontal wound healing and regeneration. However, at present there are virtually no data from clinical studies evaluating the effects of HA when used in conjunction with reconstructive periodontal surgery as compared to the use of EMD. Therefore, the aim of this randomized controlled clinical trial was to compare the clinical outcomes obtained in intrabony defects following regenerative periodontal surgery using the single flap approach (SFA) in conjunction with either HA or EMD.

Methodology
Thirty-two intrabony defects in 32 healthy subjects were randomly assigned: HA (test group) or EMD (control group). Clinical attachment level (CAL), probing depth (PD), gingival recession (REC) and bleeding on probing (BOP) were recorded at baseline,12-,18-and 24-months after surgery.

Results
At 24-months, both treatments resulted in statistically significant clinical improvements evidenced by PD-reduction and CAL-gain. The mean CAL-gain was 2.19 ± 1.11 mm in the test and 2.94 ± 1.12 mm in the control sites, respectively, without statistically significant difference between the groups. PD-reduction was statistically significantly higher for the control group (4.5 ± 0.97 mm) than the test group (3.31 ± 0.70 mm). Test sites showed slightly lower REC values (1.19 ± 0.75 mm) than the control sites (1.69 ± 0.70 mm). No statistically significant changes were observed in terms of BOP changes within and between the groups.

Conclusions
Within their limits the present findings indicate that a) **both treatments led to statistically significant long-term clinical improvements**, and b) HA appears to represent a valuable alternative for regenerative treatment in intrabony periodontal defects.

Download PDF
Healing of intrabony defects following regenerative surgery by means of single-flap approach in conjunction with either hyaluronic acid or an enamel matrix derivative: a 24-month randomized controlled clinical trial (nih.gov)

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-β1 (TGF-β1) 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**.

Eliezer M, Sculean A, Miron RJ, et al. 'Hyaluronic acid slows down collagen membrane degradation in uncontrolled diabetic rats.'J Periodontal Res. 2019;00:1–9. https://doi.org/10.1111/jre.12665

Objective
To examine the in vitro biokinetics of hyaluronic acid (HA) from a collagen membrane (CM) and to evaluate the in vivo effect of immersion of the CM in HA solution on its degradation in streptozotocin (STZ)-induced diabetes conditions in a rat calvaria subcutaneous model.

Background
CM degradation is accelerated in uncontrolled diabetic rats. Immersion of CM in HA has been suggested to decrease their resorption rate without interfering with their tissue integration and structural degradation. However, it is unknown to what extent CM degradation may be influenced by its immersion in HA solution under a condition mimicking a medically compromised situation with an increased inflammatory level such as diabetes.

Methodology
CMs were soaked in cross-linked HA. Protein adsorption and the HA release were quantified by ELISA. Diabetes was induced in sixteen rats, while 16 healthy rats served as control. CM was prepared and labeled prior to implantation with Biotin. Seventeen CM were immersed in HA and 17 CM in PBS. In each animal, one test or one control disk was implanted. In order to compare the collagen content, two similar non-implanted CM were used as baseline. Fourteen days after surgery, thirty-two animals were sacrificed. The entire calvaria including the skin above, was chemically fixed, decalcified, and embedded in paraffin. Five-µm-thick sections were analyzed histologically and histomorphometrically using H&E and avidin-peroxidase staining.

Results
The in vitro results demonstrated that the CM adsorbed roughly 80% of the total HA content. After 10 days, 36.3% of the initial HA remained on the CM. The in vivo results demonstrated that **diabetes significantly reduced the thickness of the CM, while HA had a significant effect on keeping the membrane thickness**. HA increased the residual collagen content in the diabetic group ($P < 0.0001$) but no such effect was observed in the healthy group.

Conclusion
Immersion of CM in HA prior to the implantation delays membrane degradation in uncontrolled diabetic compared with normoglycemic rats.

Pilloni A, Nardo F, Rojas MA. Surgical Treatment of a Cemental Tear-Associated Bony Defect Using Hyaluronic Acid and a Resorbable Collagen Membrane: A 2-Year Follow-Up. Clinical Advances in Periodontics, Vol. 9, No. 2, June 2019 doi: 10.1002/cap.10053

Objectives

A cemental tear (CeT) is a special type of surface root fracture that may cause periodontal and even periapical tissue destruction. Unfortunately, there is limited knowledge as to how these rare cases can effectively be treated. The present case is the first reported in the literature treating a bony defect caused by a cemental tear with hyaluronic acid (HA) and a collagen membrane. The aim of this case report is to present a regenerative surgical approach with clinical and tomographic success and stability at 2-year follow-up.

Case Presentation

A 61-year-old patient presented with spontaneous pain and gingival swelling over his right central maxillary incisor. Radiographically, a radiolucent area was observed in the medial third between both central incisors. The tomographic evaluation showed a buccal bone dehiscence and a bony defect. Once the differential diagnosis with an endodontic-periodontal lesion and root fracture was performed, CeT was the presumptive diagnosis. During the exploratory flap surgery, a small root fragment (CeT) on the mesial side of the tooth was founded and removed. The bony lesion was treated with hyaluronic acid (HA) and a resorbable collagen membrane. At 2-year follow-up clinical, radiographic, and tomographic success was observed.

Conclusion

A **CeT-associated bony defect** could be successfully treated after removing cemental fragments and performing a regenerative approach using HA and a resorbable collagen membrane.

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Surgical Treatment of a Cemental Tear-Associated Bony Defect Using Hyaluronic Acid and a Resorbable Collagen Membrane: A 2-Year Follow-Up (wiley.com)

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-ß3, 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.

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 he**aling in terms of epithelization and color match.

Akyildiz S, Soluk-Tekkesin M, Keskin-Yalcin B, Unsal G, Ozel Yildiz S, Oscan I, Cakarer S, "Acceleration of fracture healing in experimental model: Platelet-Rich Fibrin or Hyaluronic Acid?" J Craniofac Surg, 2018 oct;29(7):1794-1798. Doi: 10.1097/SCS.0000000000004934

Objective

In this study, we compared the bone-healing effects of the local application of platelet-rich fibrin (PRF) and hyaluronic acid (HA) to bilateral tibial fractures in rats.

Methodology

Twenty-three adult male Sprague- Dawley rats were used. Twenty-two animals were randomly allocated to a control group (n¼6) and 2 study groups: PRF (n¼8) and HA (n¼8). The 23rd rat was used as a donor to obtain PRF. Each group was divided into 2 subgroups for histomorphometric and radiologic assessments at 2 and 6weeks. Foreign body reaction, necrosis, inflammation, new bone formation, and fibrosis were investigated as bone healing parameters in terms of histopathologic analysis. The difference between the groups for these parameters was evaluated. The radiologic evaluation was performed by comparing the 3-dimensional reconstruction images of the fracture sites between the study and control groups.

Results

Histomorphometric evaluation showed that at 2 weeks postoperatively, the control group showed lesser bone formation (26.1; 6.6%) when compared to the study (HA: 54.7;9.7%; PRF: 75.3; 19.2%) groups and PRF group showed highest total ossification. At 6 weeks postoperatively the PRF group showed lesser total ossification (50.7; 28.2%) when compared to control (76.3; 21.7%) and HA group. **The HA (88.8; 13.3%) showed highest total ossification**. In the control group, fibrosis was more prominent at week 6, whereas in the HA and PRF groups the amount of ossification increased. In contrast to histopathologic healing, radiologic bone healing did not differ significantly among the study and control groups 2 weeks after surgery, whereas at 6 weeks, the results of radiologic bone formation were in accordance with those of histopathologic bone healing.

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.

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.

Briguglio F, Briguglio E, Briguglio R, Cafiero C, Isola G. Treatment of infrabony periodontal defects using a resorbable biopolymer of hyaluronic acid: a randomized clinical trial. Quintessence Int. 2013;44(3):231-240. doi:10.3290/j.qi.a29054

Objective
This randomized clinical study examined the use of hyaluronic acid to treat infrabony periodontal defects over a period of 24 months.

Methodology
Forty subjects with a two-wall infrabony defect (probing depth [PD] >= 7 mm; clinical attachment level [CAL] >= 7 mm) were selected. The defects were randomly divided into two groups: sites treated with hyaluronic acid (test group) and those treated with open flap debridement (control group).

Results
The 12- and 24-month evaluations were based on clinical and radiographic parameters. The primary outcome variable was CAL. Test defects shows a mean CAL gain of 1.9 ± 1.8 mm, while the control defects yielded a significantly lower gain of 1.1 ± 0.7 mm. PD reduction was also significantly higher in the test group (1.6 ± 1.2 mm) than in the control group (0.8 ± 0.5 mm). Frequency distribution analysis of the study outcomes indicated that hyaluronic acid increased the predictability of clinically significant results (CAL gains >= 2 mm and PD reduction >= 2 mm) in the test group compared with the controls.

Conclusions
The treatment of infrabony defects with hyaluronic acid offered an additional benefit in terms of **CAL gain, PD reduction, and predictability** compared to treatment with open flap debridement.

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.

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**.

Furcation

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.

Aim
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.

Materials 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 ± 1.51 mm2) and xHyA/CM (4.32 ± 1.14 mm2) groups were larger than those in the OFD (3.25 ± 0.81 mm2) and CM (3.31 ± 2.26 mm2) groups. The xHyA (6.25 ± 1.45 mm) and xHyA/CM (6.40 ± 1.35 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 ± 0.85 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.

Conclusions:
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.

Download PDF
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 (wiley.com)

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-β1 (TGF-β1) 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**.

Marin S, Popovic-Pejicic S, Radosevic-Caric B, Trtić N, Tatic Z, Selakovic S. Hyaluronic acid treatment outcome on the post-extraction wound healing in patients with poorly controlled type 2 diabetes: A randomized controlled split-mouth study. Med Oral Patol Oral Cir Bucal. 2020 Mar 1;25(2):e154-e160. doi: 10.4317/medoral.23061. PMID: 32040462; PMCID: PMC7103456.

Objective
Hyaluronic acid is widely used in the medical field. However, there is a lack of research about its effect on patients with certain risks, such as compromised wound healing commonly found in patients with poorly controlled type 2 diabetes. The aim of this study is to investigate the efficacy of hyaluronic acid on the post-extraction wound healing and pain in patients with poorly controlled type 2 diabetes.

Methodology
The randomized controlled split-mouth study was designed, which included 30 patients with poorly controlled type 2 diabetes with a bilaterally same teeth in the lower jaw for extraction. The sockets treated with 0.8% hyaluronic acid represented the study group, while the sockets where hyaluronic acid was not applied represented the control group. Wound closure rate (WCR), clinical scores in wound healing scale (WHS) and pain intensity in Visual analogue scale (VAS) were recorded. Patients were followed up on 5th, 10th, 15th, 20th, 25th day after tooth extraction.

Results
The results showed **a higher WCR at the extraction site where hyaluronic acid was applied**. Also, statistically significant difference was found ($p < 0.001$). In regard to WHS, the sockets treated with hyaluronic acid showed better healing, especially on day 10 ($p=0.006$) and day 15 ($p=0.021$). However, there were no statistically significant differences in VAS scores between groups.

Conclusion
Hyaluronic acid placed in post-extraction socket in patients with poorly controlled diabetes may **improve wound healing**, especially in the first days after application.

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-ß3, 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.

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.

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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 he**aling in terms of epithelization and color match.

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Objective

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Methodology

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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.

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 μg mL−1) in high-molecular weight-hyaluronic acid (HMW-HA), Group B; BDNF (50 μg mL−1) in HMW-HA, Group C; HMW-HA acid only, Group D; unfilled defect, or Group E; BDNF (500 μg 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 μg 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 μg 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).

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.

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.

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.

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**.

Gingival recessions

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±1.16mm vs., 0.72±0.62mm 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±1.78mm versus 2.40±1.35mm and 1.69±0.98mm versus 0.74±0.68mm, 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.

Download PDF
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)

Guldener K, Lanzrein C, Eliezer M, Katsaros C, Stähli A, Sculean 'A Treatment of Single Mandibular Recessions with the modified coronnal advanced tunnel or laterally closed tunnel, hyaluronic acid, and subepithelial connective tissue graft :a report of 12 cases.' Quintessence Int 2020;51:2-9;doi: 10.3290/j.qi.a44492

Objective

To clinically evaluate the healing of mandibular Miller Class I and II isolated gingival recessions treated with the modified coronally advanced tunnel (MCAT) or laterally closed tunnel (LCT) combined with hyaluronic acid (HA) and subepithelial connective tissue graft (SCTG).

Methodology

Twelve healthy patients exhibiting one isolated mandibular Miller Class I or II (Cairo Class 1) gingival recession of a depth of ≥ 3 mm, were consecutively treated with the MCAT or LCT in conjunction with HA and SCTG. Treatment outcomes were assessed at baseline and at least 6 months postoperatively. The primary outcome variable was complete root coverage (CRC).

Results

Postoperative pain and discomfort were low and no complications such as postoperative bleeding, allergic reactions, abscesses, or loss of SCTG occurred. After a mean follow-up of 18.9 ± 10 months, statistically significant ($P < .0001$) root coverage was obtained in all 12 defects. CRC was measured in six out of the 12 cases (50%), four cases showed a root coverage of over 95%, while the remaining two cases reached 80% and 85%. **Mean root coverage was 96.09%**. **Mean keratinized tissue width increased from 1.6 ± 0.8 mm to 4.9 ± 1.3 mm** ($P < .0001$) from baseline to follow-up, while mean probing depth showed no statistically significant changes (1.8 ± 0.9 mm vs 1.3 ± 0.5 mm).

Conclusion

Within their limits, the present results indicate that the described treatment approach may lead to **predictable root coverage of isolated mandibular Miller Class I and II** (Cairo Class 1) gingival recessions.

Lanzrein C, Guldener K, Imber JC, Katsaros C, Stähli A, Sculean A. Treatment of multiple adjacent recessions with the modified coronally advanced tunnel or laterally closed tunnel in conjunction with cross-linked hyaluronic acid and subepithelial connective tissue graft: a report of 15 cases. Quintessence Int. 2020;51(9):710-719. doi: 10.3290/j.qi.a44808. PMID: 32577705.

Objectives
To evaluate the healing of multiple adjacent type 1 and 2 gingival recessions (RT1 and RT2) treated with the modified coronally advanced tunnel (MCAT) or the laterally closed tunnel (LCT) in conjunction with a cross-linked hyaluronic acid and subepithelial palatal connective tissue grafts.

Method and materials
Fifteen healthy patients exhibiting multiple adjacent mandibular or maxillary RT1 and RT2 of a depth of ≥ 2 mm, were treated with the MCAT or LCT in conjunction with cross-linked hyaluronic acid and subepithelial palatal connective tissue grafts. Results were assessed at baseline and after a minimum of 6 months. The primary outcome variable was root coverage. Esthetic outcomes were evaluated on photographs using the root coverage esthetic score.

Results
Postoperative pain and discomfort were low and no complications occurred. Data analyses were performed at patient level. After a mean follow-up of 17 ± 5.4 months, statistically significant root coverage was obtained in all 15 cases ($P < .0001$). Complete root coverage was obtained in 3 out of 15 cases (20%). Root coverage amounted to > 95% in three patients, was between 90% and 95% in four patients, and reached 87.5% in another patient. In three further patients root coverage measured 75%, 77%, and 64.6%, respectively. Mean root coverage measured 85.1 ± 23.2%. Mean keratinized tissue width increased from 2.5 ± 1.0 mm to 3.7 ± 0.7 mm ($P < .0001$) from baseline to follow-up, while mean probing depth showed no statistically significant changes (1.3 ± 0.5 mm vs 1.5 ± 0.5 mm). The mean root coverage esthetic score was 7.9 ± 1.9, while in the three cases exhibiting complete root coverage, a maximum root coverage esthetic score (10) was given for all treated teeth.

Conclusion
Within their limits, the present results indicate that the described treatment approach may **lead to predictable root coverage of multiple mandibular** and maxillary RT1 and RT2.

Eliezer M, Sculean A, Miron RJ, et al. 'Hyaluronic acid slows down collagen membrane degradation in uncontrolled diabetic rats.'J Periodontal Res. 2019;00:1–9. https://doi.org/10.1111/jre.12665

Objective
To examine the in vitro biokinetics of hyaluronic acid (HA) from a collagen membrane (CM) and to evaluate the in vivo effect of immersion of the CM in HA solution on its degradation in streptozotocin (STZ)-induced diabetes conditions in a rat calvaria subcutaneous model.

Background
CM degradation is accelerated in uncontrolled diabetic rats. Immersion of CM in HA has been suggested to decrease their resorption rate without interfering with their tissue integration and structural degradation. However, it is unknown to what extent CM degradation may be influenced by its immersion in HA solution under a condition mimicking a medically compromised situation with an increased inflammatory level such as diabetes.

Methodology
CMs were soaked in cross-linked HA. Protein adsorption and the HA release were quantified by ELISA. Diabetes was induced in sixteen rats, while 16 healthy rats served as control. CM was prepared and labeled prior to implantation with Biotin. Seventeen CM were immersed in HA and 17 CM in PBS. In each animal, one test or one control disk was implanted. In order to compare the collagen content, two similar non-implanted CM were used as baseline. Fourteen days after surgery, thirty-two animals were sacrificed. The entire calvaria including the skin above, was chemically fixed, decalcified, and embedded in paraffin. Five-µm-thick sections were analyzed histologically and histomorphometrically using H&E and avidin-peroxidase staining.

Results
The in vitro results demonstrated that the CM adsorbed roughly 80% of the total HA content. After 10 days, 36.3% of the initial HA remained on the CM. The in vivo results demonstrated that **diabetes significantly reduced the thickness of the CM, while HA had a significant effect on keeping the membrane thickness.** HA increased the residual collagen content in the diabetic group ($P < 0.0001$) but no such effect was observed in the healthy group.

Conclusion
Immersion of CM in HA prior to the implantation delays membrane degradation in uncontrolled diabetic compared with normoglycemic rats.

Pilloni A, Schmdlin PR, Sahrmann 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 ± 13.0%for test and 73.1 ± 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.

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-ß3, 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 J, Radakovic S, Pirracchio L, Sculean A 'The clinical effect of Hyaluronic acid on root recession coverage: a case series, 6 months evaluation' PD172, Poster Presentation, EuroPerio 9, June 2018, Amsterdam

Background
The data on the potential benefits Hyaluronic acid (HA) for recession coverage surgery is still limited. Aim To evaluate the potential effects of HA on early wound healing and clinical outcomes following recession coverage surgery.

Clinical Procedure
Fifteen female patients (mean age 32.2 years) exhibiting a total of 25 single or multiple Miller Class I, II and III gingival recessions were consecutively treated by means of the modified coronally advanced tunnel (MCAT) or the laterally moved tunnel (LMT) in conjunction with the application of hyaluronic acid (Hyadent, Regedent AG, Zurich, Switzerland) on the root surfaces and subsequent placement and a palatal subepithelial connective tissue graft (SCTG). Intraoral photos were taken at baseline, 2 and 24 weeks postoperatively. Recession Coverage Healing Index (RCHI) was recorded 14 days after surgery. Recession depth (RD), recession coverage (RC), probing depth (PD), width of the attached gingiva (AG) were measured at baseline and at 24 weeks following surgery.

Outcomes
The material comprised 8 Miller Class I, 5 Miller Class II and 13 Miller Class III recessions. Four defects were located in the upper jaw, while the rest of 25 recessions were in the lower jaw. Healing was uneventful in all cases and the RCHI 14 days measured 0.18 ; 0.39. At 6 months, statistically significant improvements in RD and RC were found in all defects. RD improved from 3.55 mm ; 1.35 mm at baseline to 0.38 mm ; 0.68 mm ($p < 0.0001$) at 6 months while RC amounted to 90.03%; 17.1.

Conclusion
The present findings suggest that the use of **HA as adjunctive to recession coverage surgery** is safe and may positively influence the clinical outcomes.

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 he**aling in terms of epithelization and color match.

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

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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.

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Abstract

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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.

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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.

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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**.

Bone Augmentation

Cervino G, Meto A, Fiorillo L, Odorici A, Meto A, D'Amico C, Oteri G, Cicciù M. Surface Treatment of the Dental Implant with Hyaluronic Acid: An Overview of Recent Data. Int J Environ Res Public Health. 2021 Apr 27;18(9):4670. doi: 10.3390/ijerph18094670. PMID: 33925742; PMCID: PMC8125310.

Background

The aim of this work was to analyze the various surface treatments in titanium implants, demonstrating that the topography and surface chemistry of biomaterials can correlate with the host response; also focusing on the addition of HA to the implant surface and assessing the biological implications during early stages of recovery.

Methods

Used as a coating, HA acts on the migration, adhesion, proliferation and differentiation of cell precursors on titanium implants by improving the connection between implant and bone. Furthermore, the improvement of the bioactivity of the implant surfaces through HA could therefore facilitate the positioning of the dental prosthesis precisely in the early loading phase, thus satisfying the patients' requests.

Conclusion

Unless otherwise proven, the addition of HA to the implant surface may also play a role in the soft tissue morphology. The use of collagen molecules can favor a maturation of the alveolar tissues avoiding the possible disadvantage of transparency and therefore ofvisibility of the implant body from the mucosa. The literature shows that the presence of these modified surfaces can not only favor the healing phases, but also play a role in the management of implant pathology. Certainly, further studies are needed to confirm what was found, on a large sample. The final hypothesis should be supported by experimental studies in animals and humans to evaluate the final results and confirm the initial idea.

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IJERPH | Free Full-Text | Surface Treatment of the Dental Implant with Hyaluronic Acid: An Overview of Recent Data | HTML (mdpi.com)

Stiller M. et al. 'Performance of β-tricalcium phosphate granules and putty bone grafting materials after bilateral sinus floor augmentation in humans' Biomaterials 2014

Objective

Sinus floor augmentation (SFA) using bone grafting materials, and in particular calcium phosphates (CaP), is a well-established pre-implantology procedure. The use of CaP simplifies SFA procedures. β-tricalcium phosphate (β-TCP) is amply used for SFA. This study evaluated the clinical and osteogenic performance of β-TCP granules (TCP-G) and a β-TCP putty (TCP-P) bone graft material. TCP-P consisted of TCP-G in a hyaluronic acid (HyA) carrier. Bone formation, volume stability and osteogenic marker expression after bilateral SFA in patients was assessed.

Methodology

Eight patients were selected for a split-mouth design. Biopsies obtained six months after SFA, were processed for immunohistochemical analysis of collagen type I (Col I), alkaline phosphatase (ALP), osteocalcin (OC) and bone sialoprotein (BSP). Histomorphometric analysis determined bone, grafting material and marrow space percentages. Cone-beam computed tomography was used to calculate the graft volume and its stability.

Conclusion

Both materials allowed excellent **bone regeneration and volume stability**. TCP-P displayed better surgical handling properties, greater bone formation, higher expression of Col I, ALP, OC and BSP; as well as significantly lower grafting volume reduction values. HyA had no adverse effect on TCP-P performance. Due to its clinical and osteogenic performance, TCP-P can be regarded as excellent bone grafting material for SFA.

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-β1 (TGF-β1) 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**.

Marin S, Popovic-Pejicic S, Radosevic-Caric B, Trtić N, Tatic Z, Selakovic S. Hyaluronic acid treatment outcome on the post-extraction wound healing in patients with poorly controlled type 2 diabetes: A randomized controlled split-mouth study. Med Oral Patol Oral Cir Bucal. 2020 Mar 1;25(2):e154-e160. doi: 10.4317/medoral.23061. PMID: 32040462; PMCID: PMC7103456.

Objective
Hyaluronic acid is widely used in the medical field. However, there is a lack of research about its effect on patients with certain risks, such as compromised wound healing commonly found in patients with poorly controlled type 2 diabetes. The aim of this study is to investigate the efficacy of hyaluronic acid on the post-extraction wound healing and pain in patients with poorly controlled type 2 diabetes.

Methodology
The randomized controlled split-mouth study was designed, which included 30 patients with poorly controlled type 2 diabetes with a bilaterally same teeth in the lower jaw for extraction. The sockets treated with 0.8% hyaluronic acid represented the study group, while the sockets where hyaluronic acid was not applied represented the control group. Wound closure rate (WCR), clinical scores in wound healing scale (WHS) and pain intensity in Visual analogue scale (VAS) were recorded. Patients were followed up on 5th, 10th, 15th, 20th, 25th day after tooth extraction.

Results
The results showed **a higher WCR at the extraction site where hyaluronic acid was applied**. Also, statistically significant difference was found ($p < 0.001$). In regard to WHS, the sockets treated with hyaluronic acid showed better healing, especially on day 10 ($p=0.006$) and day 15 ($p=0.021$). However, there were no statistically significant differences in VAS scores between groups.

Conclusion
Hyaluronic acid placed in post-extraction socket in patients with poorly controlled diabetes may **improve wound healing**, especially in the first days after application.

Eliezer M, Sculean A, Miron RJ, Nemcovsky C, Bosshardt DD, Fujioka-Kobayashi M, Weinreb M, Moses O. Cross-linked hyaluronic acid slows down collagen membrane resorption in diabetic rats through reducing the number of macrophages. Clin Oral Investig. 2022 Mar;26(3):2401-2411. doi: 10.1007/s00784-021-04206-x. Epub 2021 Oct 4. PMID: 34608575.

Objective
We previously showed that accelerated degradation of collagen membranes (CMs) in diabetic rats is associated with increased infiltration of macrophages and blood vessels. Since pre-implantation immersion of CMs in cross-linked high molecular weight hyaluronic acid (xHyA) delays membrane degradation, we evaluated here its effect on the number of macrophages and endothelial cells (ECs) within the CM as a possible mechanism for inhibition of CM resorption.

Background
Diabetes was induced with streptozotocin in 16 rats, while 16 healthy rats served as control. CM discs were labeled with biotin, soaked in CLHA or PBS, and implanted under the scalp. Fourteen days later, CMs were embedded in paraffin and the number of macrophages and ECs within the CMs was determined using antibodies against CD68 and transglutaminase II, respectively.

Results
Diabetes increased the number of macrophages and ECs within the CMs (~2.5-fold and fourfold, respectively). Immersion of CMs in CLHA statistically significantly reduced the number of macrophages ($p < 0.0001$) in diabetic rats, but not that of ECs. In the healthy group, CLHA had no significant effect on the number of either cells. Higher residual collagen area and membrane thickness in CLHA-treated CMs in diabetic animals were significantly correlated with reduced number of macrophages but not ECs.

Conclusion
Immersion of CM in **xHyA inhibits macrophage infiltration and reduces CM degradation in diabetic animals**.

Clinical relevance
The combination of CLHA and CM may represent a valuable approach when guided tissue regeneration or guided bone regeneration procedures are performed in diabetic patients.

Eliezer M, Sculean A, Miron RJ, et al. 'Hyaluronic acid slows down collagen membrane degradation in uncontrolled diabetic rats.'J Periodontal Res. 2019;00:1–9. https://doi.org/10.1111/jre.12665

Objective
To examine the in vitro biokinetics of hyaluronic acid (HA) from a collagen membrane (CM) and to evaluate the in vivo effect of immersion of the CM in HA solution on its degradation in streptozotocin (STZ)-induced diabetes conditions in a rat calvaria subcutaneous model.

Background
CM degradation is accelerated in uncontrolled diabetic rats. Immersion of CM in HA has been suggested to decrease their resorption rate without interfering with their tissue integration and structural degradation. However, it is unknown to what extent CM degradation may be influenced by its immersion in HA solution under a condition mimicking a medically compromised situation with an increased inflammatory level such as diabetes.

Methodology
CMs were soaked in cross-linked HA. Protein adsorption and the HA release were quantified by ELISA. Diabetes was induced in sixteen rats, while 16 healthy rats served as control. CM was prepared and labeled prior to implantation with Biotin. Seventeen CM were immersed in HA and 17 CM in PBS. In each animal, one test or one control disk was implanted. In order to compare the collagen content, two similar non-implanted CM were used as baseline. Fourteen days after surgery, thirty-two animals were sacrificed. The entire calvaria including the skin above, was chemically fixed, decalcified, and embedded in paraffin. Five-µm-thick sections were analyzed histologically and histomorphometrically using H&E and avidin-peroxidase staining.

Results
The in vitro results demonstrated that the CM adsorbed roughly 80% of the total HA content. After 10 days, 36.3% of the initial HA remained on the CM. The in vivo results demonstrated that **diabetes significantly reduced the thickness of the CM, while HA had a significant effect on keeping the membrane thickness**. HA increased the residual collagen content in the diabetic group ($P < 0.0001$) but no such effect was observed in the healthy group.

Conclusion
Immersion of CM in HA prior to the implantation delays membrane degradation in uncontrolled diabetic compared with normoglycemic rats.

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-ß3, 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.

Bayoum, A., Nadershah, M., Albandar, A., Alsulaimani, B.S., Sankour, I., Gadi, L., Osama, O.A., Tayeb, R., Quqandi, R., Dabroom, W., & Merdad, Y. (2018). The Effect of Cross-Linked Hyaluronic Acid in Surgical Extraction of Impacted Mandibular Third Molars. International journal of dentistry and oral health, 4.

Objectives
The aim of the study was to evaluate the effectiveness of cross-linked Hyaluronic Acid (HA) gel on facial swelling, pain, and truisms after extraction of impacted mandibular third molars.

Methodology
This randomized, double-blinded, splitmouth clinical trial included 14 patients. For each patient, a combination of cross-linked HA with Gelfoam scaffold were randomly applied to one extraction site, while Gelfoam alone was applied to the other extraction site. Measurements of three facial reference points, pain and maximum mouth opening were recorded preoperatively, as well as on the 2nd, 4th and 7th days after the surgery.

Results
The scores for facial swelling, pain and trismus were the highest on the 2nd postoperative day and decreased gradually on the 4th to the 7th days in both groups. Cross-linked HA group demonstrated statistically significant reduction in swelling, pain, and trismus on the 7th postoperative day when compared to its control group ($p<0.05$).

Conclusion
The application of cross-linked HA after extraction of impacted mandibular third molars has **a positive impact on postoperative swelling, pain and trismus** after the extraction of impacted lower third molars.

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 he**aling in terms of epithelization and color match.

Alcantara CEP et al 'Hyaluronic acid accelerates bone repair in human dental sockets: a randomized triple-blind clinical trial' Braz Oral Res. 2018.DOI 10.1590/1807-3107bor-2018.vol32.0084

Objective
This study evaluated the effects of hyaluronic acid (HA) on bone repair of human dental sockets.

Methodology
Thirty-two lower first premolars were extracted from 16 patients (2 per patient) for orthodontic reasons. Following the extractions, one socket was randomly filled with 1% HA gel, while the other was allowed to naturally fill with blood clot. After 30 and 90 days of surgery, patients underwent cone beam computed tomography. Five central orthoradial slices were captured from each socket. The gray intensity was measured in each image and results were reported as mean percentage of bone formation. The buccolingual alveolar ridge width was measured and dimensional changes were compared between the postoperative int ervals. The pattern of alveolar trabecular bone was evaluated through the fractal dimension.

Results
Treated **sockets showed a higher percentage of bone formation** and fractal dimension values (58.17% and 1.098, respectively) compared with controls (48.97% and 1.074, respectively) in the 30-day postoperative period ($p < 0.05$). After 90 days, there was no significant difference between groups. Additionally, no significant difference was found between groups regarding the alveolar dimensions ($p > 0.05$).

Conclusion
Use of 1% **HA gel after tooth extraction accelerates bone repair** in human dental sockets.

Kim JJ, Song HY, Ben Amara H, Kyung-Rim K, Koo KT. Hyaluronic Acid Improves Bone Formation in Extraction Sockets With Chronic Pathology: A Pilot Study in Dogs. J Periodontol. 2016;87(7):790-795. doi:10.1902/jop.2016.150707

Background
Previous studies on ridge preservation focusing on fresh extraction sockets using graft materials for ridge preservation procedures have reported a delay in the tissue modelling and remodelling phases. The objective of this study is to evaluate the effect of hyaluronic acid (HA) on healing of infected sockets.

Methodology
Six beagle dogs were used in this study. Both mandibular third premolars were hemisected, and the distal roots were extracted. Subsequently, periodontal and endodontic lesions were induced at the remaining mesial root. After communication of the periodontal lesion, an endodontic periapical lesion was observed at 4 months, and the mesial roots of both the right and left sides were extracted. HA was applied into the socket of the test group, and no treatment was administered to the other group (control group). Three months after extraction of the mesial roots, the dogs were sacrificed, and histologic evaluations were performed.

Results
The sockets were filled by mineralized bone (47.80% ± 6.60%) and bone marrow (50.47% ± 6.38%) in the control group, whereas corresponding values were 63.29% ± 9.78% and 34.73% ± 8.97% for the test group, respectively. There was a statistically significant difference between the groups. Reversal lines and a copious lineup of osteoblasts were observed in the middle and apical parts of the sockets in the test group.

Conclusion
An infected socket shows delayed healing of the socket wound, and **HA, because of its osteoinductive, bacteriostatic, and anti-inflammatory properties, may improve bone formation and accelerate wound healing in infected sockets.**

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.

ELkarargy A. Alveolar Sockets Preservation Using Hydroxyapatite / Beta tricalcium Phosphate with Hyaluronic Acid (Histomorphometric study). J Am Sci 2013; 9(1): 556-563]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 78

Objective

Alveolar atrophy following tooth extraction remains a challenge for future dental implant placement. Immediate implant placement and postextraction alveolar preservation are two methods that are used to prevent significant postextraction bone loss. The purpose of this study is to investigate the usefulness of hydroxyapatite / beta tricalcium phosphate (HA/BTCP) with hyaluronic acid (HY) for alveolar sockets preservation.

Methodology

Thirty-two New Zealand white rabbits were subjected to the lower left incisor extraction. The rabbits were equally divided into three groups. The extracted sockets (n = 12/group) were filled with: HA/BTCP, HA/BTCP + HY and blood clot (control). All rabbits were sacrificed for histological and histomorphometric evaluation after 4- and 8-week healing periods. .

Results

The results demonstrated that all sites examined in this study displayed evidence of **new bone formation**. A statistically significant difference in the amount of new bone formation were found between sites that healed for an average of 8 only. The results demonstrating approximately 78%,68 % and 63% of new vital bone formation for groups grafted with HA/BTCP +HY , HA/BTCP and control group respectively after 8 weeks postoperatively.

Conclusion

In conclusion these results exhibited that the use of hydroxyapatite / beta tricalcium phosphate with hyaluronic acid appears to be more efficient in **osteoconduction** when compared with of hydroxyapatite / beta tricalcium phosphate alone and could be promising strategy for preservation of alveolar sockets.

Download PDF

Alveolar-Sockets-Preservation-Using-Hydroxyapatite-Beta-tricalcium-Phosphate-with-Hyaluronic-Acid-Histomorphometric-study.pdf (researchgate.net)

Mendes RM et al. 'Sodium hyaluronate accelerates the healing process in tooth sockets of rat' Arch Oral Biol 2008;53:1155–1162

Objective

In this study we evaluated the effects of sodium hyaluronate (HY) in the healing process of tooth sockets of rats.

Methodology

Immediately after the extraction of the upper first molars of male Holtzman rats, right sockets were treated with 1% HY gel (0.1 ml), while left sockets were used as control (blood clot). The animals were sacrificed at 2, 7, and 21 days after tooth extraction and upper maxillaries processed for histological and morphometric analysis of the apical and medium thirds of the sockets. Carbopol, an inert gel, was used to evaluate the mechanical effect of gel injection into sockets. Expression of bone morphogenetic protein-2 (BMP-2) and osteopontin (OPN) was determined by immunohistochemistry at 1, 2, 3, 4, 5, and 7 days after tooth extraction.

Results

Histological analysis showed that HY treatment induced **earlier trabecular bone deposition resulting in a bone matrix more organized** at 7 and 21 days after tooth extraction. Also, HY elicited significant increase in the amount of bone trabeculaes at 7 and 21 days after tooth extraction (percentage of trabecular bone area at 7 days: 13.21" 4.66% vs. 2.58" 1.36% in the apical third of control sockets) and in the vessels counting at 7 days. Conversely, the number of cell nuclei was decreased in HY-treated sockets. Additionally, expression of BMP- 2 and OPN was enhanced in HY-treated sockets compared with control sockets.

Conclusion

These findings suggest that HY **accelerates the healing process in tooth sockets** of rats stimulating the expression of osteogenic proteins.

Muzaffer A. et al. 'The Effect of Hyaluronic Acid-supplemented Bone Graft in Bone Healing: Experimental Study in Rabbits' J Biomater Appl 2006 20:209

Objective

Hyaluronic acid (HA) is one of the essential components of extracellular matrix, which plays a predominant role in tissue morphogenesis, cell migration, differentiation, and adhesion. Bone allografts are frequently used to repair and reconstruct bone defects.

Methodology

In this study, two cavities of 3 mm diameter and depth have been created in the right tibia of 30 mature rabbits in accordance with the principles of general surgery. One of the cavities in the tibia is filled with HA and bovine bone graft and the other is filled with only spongiosal bone graft, for the purpose of control. On the 20th, 30th, and 40th days, rabbits have been sacrificed in equal numbers and defective regions have been extracted. The Kruskal–Wallis test was applied to the data obtained in the result of histopathologic survey of specimens.

Conclusion

The cavities that have been filled with **HA and bone graft have shown higher scores** than the control group during every period of the study.

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.

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**.

Shamma MM, Ayad SS, El-dibany RM, Nagui DA 'Evaluation of the effect of hyaluronic acid mixed with biphasic calcium phosphate on bone healing around dental implants' Alexandria Dental Journal. (2017) Vol.42 Pages:104-11

Introduction
Biphasic calcium phosphate (BCP) is very widely used as a grafting material around dental implants. The properties of such material can be enhanced by adding interpositional graft materials to enhance osteoinduction. Hyaluronic acid (HyA) is an example of osteopromoting materials that can be added to the BCP to enhance its osteoinductive properties.

Objectives
Histological evaluation of using HyA with BCP on bone healing around dental implants.

Methodology
This study was a split mouth design. It was conducted on 9 mongrel dogs. The dogs were allocated into two groups: Group A (Study Group): The right side of the mandible received dental implants with biphasic calcium phosphate bone graft mixed with hyaluronic acid following extraction of the mandibular third premolar. Group B (Control Group): The left side of the mandible received dental implants with biphasic calcium phosphate bone graft only following extraction of the mandibular third premolar. Dogs were sacrificed at 2, 4 and 6 weeks postoperatively. Segments containing the implant and bone graft were retrieved with adjacent bone to be prepared for histological examination using Haematoxylin and eosin stain and Trichrome stain.

Results
All animals survived well, and remained active and alert all over the course of the experiment. Both groups were characterized by **new bone formation**. The newly formed bone was more evident in association with group (A).

Conclusion
HyA **accelerates the onset of new bone formation** when combined with BCP for bone augmentation in the treatment of osseous defects.

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article_57868_c2494610021cdb52f296efa1c64c66d0.pdf (ekb.eg)

Ghada Bassiouny A. 'Bioinspired Approach for Dental Implant Fuctionalization: An Experimental Study Evaluating the Effect of Hyaluronate as Bioactive Implant Coating.' J Am Sci 2013;9(11):187-192]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 25

Objective
Limited osseointegration of dental implants in areas of poor quantity and quality of bone underscore the need for novel approaches that modulate host cell-implant responses to enhance osseointegration. Bioinspired strategies have emerged and included functionalizing implants with extracellular matrix proteins to augment the biological performance of dental implant. The purpose of this study was to investigate whether coating implant surface with hyaluronate will improve osseointegration compared to uncoated implant surface.

Methodology
Twelve mature New Zealand white rabbits weighing 2.5 - 3.5 kg were implanted with a hyaluronate -coated implant in one tibia and uncoated implant in the other one. Six animals were evaluated by scanning electron microscope for a period of 4 or 8 weeks.

Results
Scanning electron microscopy analysis demonstrated that the implants with hyaluronate coating had significantly the least percentage of gap distance at 8 weeks (P=0.0079) compared with the uncoated implants.

Conclusion
Biofunctionalization of the implant surface with hyaluronate significantly **improves bone to implant contact and osseointegration.**