# SCIENTIFIC EVIDENCE OF CROSS-LINKED HYALURONIC ACID GEL (xHyA) IN DENTAL REGENERATION

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# Relevant Studies on cross-linked hyaluronic acid (xHyA)

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 Nov;24(11):3923-3937. doi: 10.1007/s00784-020-03259-8. Epub 2020 Mar 31. PMID: 32236725.

**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**.

Asparuhova M, Kiryak D, Eliezer M, Mihov D, Sculean A. 'Activity of two hyaluronan preparations on primary human oral fibroblasts'. J Periodontal Res. 2019 Feb;54(1):33-45. doi: 10.1111/jre.12602. Epub 2018 Sep 27. PMCID: PMC6586051. PMID: 30264516.


**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.

**Material and Methods**

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 behavior, wound healing-related gene expression and activation of signaling kinases were analyzed by qRT-PCR and immunoblotting, respectively.

**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-β3, 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 signaling 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.

Bilhan H, Friedmann A. 'The "Tunneled Sandwich" Technique for Preserving the Buccal Tissue Volume After Immediate Implantation: A Retrospective Report of 10 Cases'. Int J Periodontics Restorative Dent. 2023 Oct 24:(7):s53-s64. doi: 10.11607/prd.6205. PMID: 37294593.


**Background**
Tooth loss or extraction is associated with significant reduction in alveolar ridge volume, extensively expressed in the anterior zone, and immediate implant placement is insufficient to overcome this problem. The proposed approach combined immediate implant placement with buccal tissue enhancement by applying a crosslinked collagen matrix hydrated with crosslinked hyaluronic acid (xHyA).

**Materials and Methods**
All 10 cases presented with a retained but narrow buccal socket wall, so immediate implant placement with the "tunneled sandwich" technique was performed after tooth extraction. The tunneled sandwich technique helped create a subperiosteal pouch for insertion of the collagen matrix buccal to the alveolar bone crest. The implants healed transmucosally by receiving either a gingiva former or an immediate temporary restoration.

**Results**
Ten sites in 10 patients demonstrated **stable, noninflamed peri-implant conditions and suitable ridge volume** at the implant neck and achieved **high pink esthetic** scores 6 months after implant loading.

**Conclusions**
The tunneled sandwich technique is a **suitable method to preserve buccal volume**, which biologically and esthetically contributes to favorable long-term results.

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; 14(22): 6795. doi: 10.3390/ma14226795. PMCID: PMC8624958. PMID: 34832196.


**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 (HA) and deproteinized porcine bone mineral.

**Methods**
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.

**Results**
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%.

**Conclusions**
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**.

Çankaya ZT, Gürbüz S, Bakirarar B, Kurtiş B. 'Evaluation of the Effect of Hyaluronic Acid Application on the Vascularization of Free Gingival Graft for Both Donor and Recipient Sites with Laser Doppler Flowmetry: A Randomized, Examiner-Blinded, Controlled Clinical Trial'. Int J Periodontics Restorative Dent. 2020 Mar/Apr;40(2):233-243. doi: 10.11607/prd.4494. PMID: 32032408.

**Objective**
This study aimed to evaluate with laser Doppler flowmetry (LDF) the effect of topical hyaluronic acid (HA) application on the vascularization of free gingival graft (FGG) donor and recipient sites during the early wound healing period and to investigate the effect of HA application on the dimensional change of the FGG.

**Methodology**
Forty systemically healthy, nonsmoking patients who required FGG due to insufficient amount of attached gingiva in a partial edentulism were randomly assigned to a study group: test (FGG+HA) or control (FGG alone). The LDF values of the donor and recipient sites were measured in both groups before the operation and at 4, 7, 10, 14, and 30 postoperative days. LDF measurement of the graft was performed as soon as the graft was taken from the palatal site. FGG dimensions (width, height, and thickness) were assessed and recorded at baseline and on day 30, as well as the percentage of the changes in these values.

**Results**
LDF values of the recipient site in the FGG+HA group were found to be statistically higher than those in the control group on days 4 and 7 (P = .013 and P = .020, respectively); however, no differences were found for days 10, 14, and 30. Additionally, no differences were found for the LDF values of the palatal site between the FGG+HA and control groups (P > .05) at all examined time points. The height of the graft measured on day 30 was statistically higher in the FGG+HA than the control group (P < .001). The percentage change in thickness and height of the FGG was statistically lower in the FGG+HA than control group (P = .028 and P < .001, respectively).

**Conclusion**
**Application of HA on the recipient bed under the FGG at the first week of healing allows the formation of a well-vascularized layer**, which acts as a barrier against tissue tensions by functioning as a scaffold between the recipient bed and FGG, thus **reducing the shrinkage of the graft**, especially in the vertical direction. This study further showed that the graft taken from the donor site had a remaining blood perfusion value of its own.

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 May; 18(9): 4670. doi: 10.3390/ijerph18094670. PMCID: PMC8125310. PMID: 33925742.

**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.

**Conclusions**
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 of visibility 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.

Diehl D, Friedmann A, Liedloff P, Jung RM, 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(19), 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.

Eliezer M, Sculean A, Miron RJ, et al. 'Hyaluronic acid slows down collagen membrane degradation in uncontrolled diabetic rats'. J Periodontal Res. 2019 Dec;54(6):644-652. doi: 10.1111/jre.12665. Epub 2019 Jun 12. PMID: 31190426.


**Aim**

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.

**Materials and Methods**

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.

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. PMCID: PMC5240222. PMID: 28093072.

**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.

**Methods**
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.

Guldener K, Lanzrein C, Eliezer M, Katsaros C, Stähli A, Sculean A. 'Treatment of single mandibular recessions with the modified coronally advanced tunnel or laterally closed tunnel, hyaluronic acid, and subepithelial connective tissue graft: a report of 12 cases'. Quintessence Int. 2020;51(6):456-463. doi: 10.3290/j.qi.a44492. PMID: 32368762.

**Objectives**
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).

**Method and materials**
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.

Hakki SS, Bozkurt SB, Sculean A, Božić D. 'Hyaluronic acid enhances cell migration, viability, and mineralized tissue-specific genes in cementoblasts'. J Periodontal Res. 2023 Dec 9. doi: 10.1111/jre.13201. Online ahead of print. PMID: 38069670.

**Background/objectives**
It has been repeatedly demonstrated that cementum formation is a crucial step in periodontal regeneration. Hyaluronic acid (HA) is an important component of the extracellular matrix which regulates cells functions and cell-cell communication. Hyaluronic acid/derivatives have been used in regenerative periodontal therapy, but the cellular effects of HA are still unknown. To investigate the effects of HA on cementoblast functions, cell viability, migration, mineralization, differentiation, and mineralized tissue-associated genes and cementoblast-specific markers of the cementoblasts were tested.

**Materials and methods**
Cementoblasts (OCCM-30) were treated with various dilutions (0, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128) of HA and examined for cell viability, migration, mineralization, and gene expressions. The mRNA expressions of osteocalcin (OCN), runt-related transcription factor 2 (Runx2), bone sialoprotein (BSP), collagen type I (COL-I), alkaline phosphatase (ALP), cementum protein-1 (CEMP-1), cementum attachment protein (CAP), and small mothers against decapentaplegic (Smad) -1, 2, 3, 6, 7, β-catenin (Ctnnb1) were performed with real-time polymerase chain reaction (RT-PCR). Total RNA was isolated on days 3 and 8, and cell viability was determined using MTT assay on days 1 and 3. The cell mineralization was evaluated by von Kossa staining on day 8. Cell migration was assessed 2, 4, 6, and 24 hours following exposure to HA dilutions using an in vitro wound healing assay (0, 1:2, 1:4, 1:8).

**Results**
At dilution of 1:2 to 1:128, HA importantly increased cell viability ($p < .01$). HA at a dilution of 1/2 increased wound healing rates after 4 h compared to the other dilutions and the untreated control group. Increased numbers of mineralized nodules were determined at dilutions of 1:2, 1:4, and 1:8 compared with control group. mRNA expressions of mineralized tissue marker including COL-I, BSP, RunX2, ALP, and OCN significantly improved by HA treatments compared with control group both on 3 days and on 8 days ($p < .01$). Smad 2, Smad 3, Smad 7, and β-catenin (Ctnnb1) mRNAs were up-regulated, while Smad1 and Smad 6 were not affected by HA administration. Additionally, HA at dilutions of 1:2, 1:4, and 1:8 remarkably enhanced CEMP-1 and CAP expressions in a dilution- and time-dependent manner ($p < .01$).

**Conclusions**
The present results have demonstrated that HA affected the expression of both mineralized tissue markers and cementoblast-specific genes. **Positive effects of HA on the cementoblast functions demonstrated that HA application may play a key role in cementum regeneration**.

Husseini B, Friedmann A, Wak R, Ghosn N, Khoury G, El Ghoul T, Abboud CK, Younes R. 'Clinical and radiographic assessment of cross-linked hyaluronic acid addition in demineralized bovine bone based alveolar ridge preservation: A human randomized split-mouth pilot study'. J Stomatol Oral Maxillofac Surg. 2023 Sep;124(4):101426. doi: 10.1016/j.jormas.2023.101426. Epub 2023 Feb 16. PMID: 36801259.

**Purpose**

To investigate clinically and radiographically at 4 months post-operatively the outcomes of mixing demineralized bovine bone material (DBBM) with cross-linked hyaluronic acid in alveolar ridge preservation.

**Material and Methods**

Seven patients presenting bilateral hopeless teeth (14 teeth) were enrolled in the study, the test site contained demineralized bovine bone material (DBBM) mixed with cross-linked hyaluronic acid (xHyA) while the control site contained only DBBM. 4 months post-operatively prior to implant placement a Cone beam computed tomography (CBCT) scan was recorded and compared to the initial scan to assess the volumetric and linear bone resorption that occurred in both sites. Clinically, sites that needed further bone grafting at the implant placement stage were recorded. Differences in volumetric and linear bone resorption between both groups were assessed using Wilcoxon signed rank test. McNemar test was also used to evaluate difference in bone grafting need between both groups.

**Results**

All sites healed uneventfully, volumetric and linear resorption differences between the baseline and 4 months post-operatively were obtained for each site. The mean volumetric and linear bone resorption were respectively 36.56 ± 1.69%, 1.42 ± 0.16 mm in the controls sites and 26.96 ± 1.83%; 0.73 ± 0.052 mm in the tests sites. The values were significantly higher among controls sites (P=0.018). No significant differences were observed in the need for bone grafting between both groups.

**Conclusion**

**Cross-linked hyaluronic acid (xHyA) appears to limit the post-extractional alveolar bone resorption when mixed with DBBM**.

Iaria R, Bortolotti G, Giovannacci I, Meleti M, Vescovi P. 'Gestione con laser Nd:YAG (1064 nm) di lesione displasica della guancia: Case report'. [Nd:YAG (1064 nm) laser management of dysplastic lesion of the cheek: case report]. Doctor Os. 2023 Jul;XXXIV 06:30-33

**Background**

We report the case of an **erythroleukoplakia** measuring 3 cm x 2.5 cm localized at the level of the left genial mucosa in a 68-year-old male patient previously treated for squamous cell carcinoma in the same region. The presence of the lesion is associated with mild symptoms of discomfort and burning upon palpation.

**Material and Methods**

After local-regional anesthesia, the profile drawing of the area to be removed was performed, followed by excision of the lesion with safety margins of 1 cm in extension and thickness. The surgery was performed using Nd:YAG laser. Subsequently, a gel based on **cross-linked hyaluronic acid** (composition: 1.6% cross-linked hyaluronic acid and 0.2% native hyaluronic acid) was applied, protected by the positioning of an absorbable porcine pericardial membrane, fixed to the edges of the surgical wound with absorbable stitches. At the end of the surgery, laser photobiostimulation (LLLT) was performed using diode lasers. The laser application was repeated on a weekly basis for the following 4 weeks.

**Results**

One week after the operation it was possible to observe good re-epithelialisation of the surgical site with total absence of signs of infection and inflammation. At two months there was **complete healing of the site**, with a slight cicatricial retraction, which was progressively reduced (as visible in the four-month check-up).

Kauffmann F, Fickl S, Sculean A, Fischer KR, Friedmann A. 'Alveolar ridge alterations after lateral guided bone regeneration with and without hyaluronic acid: a prospective randomized trial with morphometric and histomorphometric evaluation'. Quintessence Int. 2023 Oct 19;54(9):712-722. doi: 10.3290/j.qi.b4171703. PMID: 37345441.


**Objective**
To clinically and histologically evaluate the potential effect of a cross-linked, high molecular weight hyaluronic acid (xHyA) on the outcomes of guided bone regeneration performed with a demineralized bovine bone mineral (DBBM) covered with a natural collagen membrane.

**Methods and materials**
Eleven patients (eight females and three males, mean age 53 years) with a total of 27 surgical sites were treated. Treatments were performed with either DBBM and natural collagen membrane fixed with tacks (group A) or DBBM mixed with xHyA and subsequently covered with natural collagen membrane (group B). Clinical evaluations were made at baseline (T1), immediately after guided bone regeneration (T2), and at the time of implant placement (T3). Additionally, at the time of implant placement, core biopsies were retrieved and submitted for histologic analysis.

**Results**
Healing was uneventful in all cases. At 6 months, group B revealed a statistically significantly **higher crestal ridge dimension** compared to group A (P = .007). The histologic analysis revealed a tendency for **greater mineralized tissue formation** in group B compared to group A (67.5% versus 41.6%) and contained a **higher amount of new bone** (37.2%) and less DBBM residues (20.9%) than group A (12.8% new bone and 28.8% DBBM residues, respectively).

**Conclusions**
Within their limits, the present data indicate that, **during guided bone regeneration with natural collagen membrane, the combination of DBBM and xHyA may improve the quality and quantity of bone formed with DBBM alone**.

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.

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'. Clin Oral Investig. 2017 May;21(4):1013-1019. 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.

**Materials and methods**
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.

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 (Basel). 2022 Sep 20;15(19):6528. doi: 10.3390/ma15196528. PMCID: PMC9572243. PMID: 36233870.


**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.

Olszewska-Czyz I, Kralik K, Prpic J. 'Biomolecules in Dental Applications: Randomized, Controlled Clinical Trial Evaluating the Influence of Hyaluronic Acid Adjunctive Therapy on Clinical Parameters of Moderate Periodontitis'. Biomolecules. 2021 Oct 9;11(10):1491. doi: 10.3390/biom11101491. PMCID: PMC8533205. PMID: 34680123.

**Introduction**
The biological activity of hyaluronic acid (HA) has been well-researched during the past decades; however, there are few randomized, controlled trials of its clinical effects in periodontal therapy.

**Objectives**
The purpose of this study was to evaluate the effect of hyaluronic acid on the principal parameters of periodontal healing. A specific, commercially available formulation designed and registered for professional dental application, composed of 16 mg/mL of cross-linked and 2 mg/mL of non-cross-linked HA, was used as an adjunctive to non-surgical periodontal therapy, and clinical parameters were evaluated after 3 months.

**Results**
The addition of HA to periodontal therapy demonstrated more favorable clinical results regarding reduction in inflammation, measured by bleeding on probing (-6% compared to the control group) and gain in periodontal attachment (1 mm more than control group), while it had no effect on probing depth reduction. No side effects were reported.

**Conclusion**
Our study demonstrated that HA is a safe and easy-to-use biological agent; due to its wide array of properties, it may significantly improve the results of periodontal therapy. However, more long-term studies are needed to investigate whether these favorable effects remain over time.

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. PMCID: PMC8342388. PMID: 33565017


**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.

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'. Clin Adv Periodontics. 2019 Jun;9(2):64-69. doi: 10.1002/cap.10053. Epub 2019 Feb 6. PMID: 31498568.

**Introduction**
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.

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'. Clin Oral Investig. 2019 Mar;23(3):1133-1141. doi: 10.1007/s00784-018-2537-4. Epub 2018 Jun 30. PMID: 29961138.

**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.

**Material and methods**
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.

Ramanauskaite E, Machiulskiene V, Shirakata Y, Dvyliene UM, Nedzelskiene I, Sculean A. 'Clinical evaluation of sodium hypochlorite/amino acids and cross-linked hyaluronic acid adjunctive to non-surgical periodontal treatment: a randomized controlled clinical trial'. Clin Oral Investig. 2023 Nov;27(11):6645-6656. doi: 10.1007/s00784-023-05271-0. Epub 2023 Sep 23. PMCID: PMC10630230. PMID: 37740107.

**Objectives:** To compare the clinical outcomes obtained with either mechanical subgingival debridement in conjunction with a sodium hypochlorite and amino acids containing gel followed by subsequent application of a cross-linked hyaluronic acid gel (xHyA) gel, or with mechanical debridement alone.

**Materials and Methods:** Fourty-eight patients diagnosed with stages II-III (Grades A/B) generalised periodontitis were randomly treated with either scaling and root planing (SRP) (control) or SRP plus adjunctive sodium hypochlorite/amino acid and xHyA gels (test). The primary outcome variable was reduction of probing depth (PD), while changes in clinical attachment level (CAL), bleeding on probing (BOP) and plaque index (PI) were secondary outcomes. The outcomes were assessed at baseline, at 3 and 6 months following therapy.

**Results:** All patients completed the 6 months evaluation. At 6 months, the test group showed statistically significantly better results in terms of mean PD reduction (2.9 ± 0.4 vs 1.8 ± 0.6 mm, $p < 0.001$). Similarly, mean CAL gain was statistically higher in the test group compared to the control one (test: 2.6 ± 0.5 vs control: 1.6 ± 0.6 mm, $p < 0.001$). Mean BOP decreased from 81.8 ± 16.2% to 48.9 ± 14.5% in control ($p < 0.001$) and from 83.2 ± 15.5% to 17.6 ± 11.5% in test ($p < 0.001$) groups with a statistically significant difference favouring the test group ($p < 0.001$). Mean PI scores were reduced statistically significantly in both groups (from 38.8 ± 26% to 26.5 ± 20.5% in control ($p = 0.039$) and from 60.6 ± 10.9% to 12.7 ± 8.9% in test group ($p < 0.001$)), with a statistically significant difference between the groups ($p < 0.001$). The number of moderate pockets (4–6 mm) were reduced from 1518 (41.2%) to 803 (22.6%) in the control and from 1803 (48.6%) to 234 (7.7%) in the test group with a statistically significant difference between the groups ($p < 0.001$), while the number of deep pockets (≥ 7 mm) changed from 277 (7.6%) to 35 (1.0%) in the control and from 298 (8.7%) to 4 (0.1%) in test group ($p = 0.003$).

**Conclusion:** Within their limits the present data indicate that: a) both treatments resulted in statistically significant improvements in all evaluated clinical parameters, and b) the **adjunctive subgingival application of sodium hypochlorite/amino acid and xHyA to SRP yielded statistically significantly higher improvements compared to SRP alone**.

**Clinical relevance:** The combination of sodium hypochlorite/amino acid and xHyA gels to subgingival mechanical debridement appears to represent a valuable approach to additionally improve the outcomes of non-surgical periodontal treatment.

Ramanauskaite E, Machiulskiene V, Dvyliene UM, Eliezer Meizi, Sculean A. 'Clinical Evaluation of a Novel Combination of Sodium Hypochlorite/Amino Acid and Cross-linked Hyaluronic Acid Adjunctive to Non-surgical Periodontal Treatment: A Case Series'. Oral Health Prev Dent. 2023 Jul 13;21(1):279-284. doi: 10.3290/j.ohpd.b4347453. 21. 279-284. 10.3290/j.ohpd.b4347453. PMID: 37724897.

**Objective**
The adjunctive subgingival application of sodium hypochlorite/amino acid and a mixture of natural and cross-linked hyaluronic acid gels (high molecular weight) has been recently proposed as a novel modality to enhance the outcomes of non-surgical periodontal therapy. The aim of this prospective case series was to evaluate the clinical outcomes obtained following the subgingival application of a combination of sodium hypochlorite/amino acid and a mixture of natural and cross-linked hyaluronic acid (high molecular) gels in conjunction with non-surgical periodontal therapy.

**Material and Methods**
Twenty-one systemically healthy, non-smoking patients diagnosed with stage II-III, grade A/B periodontitis underwent full-mouth subgingival debridement (SD) performed with ultrasonic and hand instruments. All sites with probing depths (PD) ≥ 4 mm were treated with additional repeated (i.e., 2-3 times) instillation of sodium hypochlorite/amino acid gel in the periodontal pockets prior to and during SRP. Following mechanical debridement, a mixture of natural and cross-linked hyaluronic acid (high molecular) gel was applied in the pockets. The primary outcome variable was PD reduction; changes in clinical attachment level (CAL) and bleeding on probing (BOP) were the secondary outcomes. The clinical parameters were assessed at baseline, 3 and 6 months after therapy.

**Results**
Compared to baseline, a statistically significant mean reduction of PD values was obtained after 3 and 6 months, amounting to 2.6 ± 0.4 mm, and 2.9 ± 0.4 mm, respectively ($p < 0.001$). **Mean CAL gain measured 2.3 ± 0.5 mm at 3 months and 2.6 ± 0.5 mm at 6 months in comparison to baseline** ($p < 0.001$). Mean reduction of BOP values was 54.9 ± 16.9 % at 3 months and 65.6 ± 16.4 % at 6 months ($p < 0.001$). The number of moderate pockets (4-5 mm) decreased from 1808 at baseline to 274 at the 6-month evaluation, and the number of deep (≥ 6 mm) pockets dropped from 319 to 3, respectively.

**Conclusion**
The combination of sodium hypochlorite/amino acid and a mixture of natural and cross-linked hyaluronic acid (high molecular) adjunctive to subgingival debridement may represent a valuable approach to improve the outcomes of non-surgical periodontal treatment.

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.

**Materials and Methods**
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.

Shirakata Y, Nakamura T, Setoguchi F, Imafuji T, Shinohara Y, Matsumura S, Iwata M, Noguchi K, Ramanauskaite E, Sculean A. Histological evaluation of nonsurgical periodontal treatment with and without the use of sodium hypochlorite / amino acids and cross-linked hyaluronic acid gels in dogs. Clin Oral Investig. 2024 Apr 27;28(5):281. doi: 10.1007/s00784-024-05674-7. PMID: 38676852; PMCID: PMC11055767.

**Objectives**
To evaluate periodontal wound healing following scaling and root planing (SRP) in conjunction with the application of sodium hypochlorite/amino acids and cross-linked hyaluronic acid (xHyA) gels in dogs.

**Materials and methods**
In four beagle dogs, 2-wall intrabony defects were created and metal strips were placed around the teeth. Clinical parameters were measured 4 weeks after plaque accumulation. The experimental root surfaces were subjected to SRP with either the subgingival application of a sodium hypochlorite/amino acid gel and a xHyA gel (test group) or SRP alone (control group) using a split-mouth design. Clinical parameters were re-evaluated at 6 weeks. The animals were sacrificed at 8 weeks for histological analysis.

**Results**
The test group showed significant improvements in all clinical parameters compared to the control group. Histologically, the test group exhibited statistically significantly greater new bone formation [i.e., length of newly formed bone, new bone area] compared with the control group ($p < 0.05$). Furthermore, statistically significantly greater formation of new attachment [i.e., linear length of new cementum adjacently to newly formed bone with inserting collagen fibers] and new cementum was detected in the test group compared with the control group at 8 weeks ($p < 0.05$ and $p < 0.01$, respectively).

**Conclusion**
The adjunctive subgingival application of sodium hypochlorite/amino acid and xHyA gels to SRP offers an innovative novel approach to enhance periodontal wound healing/regeneration.

**Clinical relevance**
The present findings have for the first-time shown histologic evidence for periodontal regeneration in support of this novel treatment modality.

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. PMCID: PMC9796036. 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.

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 Mar 3;52(4):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 = Geistlich Fibro Gide®) on the periodontal wound healing/regeneration in intrabony defects.

**Method and materials**
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 histologic 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**.

Shirakata Y, Nakamura T, Kawakami Y, Imafuji T, Shinohara Y, Noguchi K, Sculean A. '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'. J Clin Periodontol. 2021 Apr;48(4):570-580. doi: 10.1111/jcpe.13433. Epub 2021 Feb 10. PMCID: PMC8248173. PMID: 33513277.

**Aim**
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).

**Materials 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 ($P<0.05$)).

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

Taman R, Fahmy M, Karam S, EL Ashwah A. 'Post-extraction socket preservation with autogenous bone graft and hyaluronic acid followed by delayed implant placement'. Alexandria Dental Journal. (2017) Vol.42 Pages:170-176.


**Objectives**

Alveolar ridge atrophy following tooth extraction remains a challenge for future implant placement. Post-extraction socket preservation and implant placement are two methods that are used to prevent significant post-extraction bone loss. The aim of this study was to evaluate the role of hyaluronic acid when mixed with autogenous bone graft in alveolar socket preservation for future implant placement.

**Materials and Methods**

A split mouth randomized clinical trial was carried out in 10 patients, 20 mandibular extraction sockets of single rooted teeth with age ranged between 25-55 years, 10 sockets were grafted with autogenous bone graft only using Auto-Max™ bone harvester and the other 10 sockets were grafted with autogenous bone graft mixed with hyaluronic acid (Hyadent™). All sockets were evaluated clinically, radiographically, and histologically (after 2 months, core biopsy was taken before implant placement) then histomophometric analysis and delayed implant insertion were done followed by implant stability assessment. After 4 months, final prosthesis was delivered.

**Results**

Histological evaluation revealed rapid thick bone deposition with many well organized osteocytes as well as osteoblast lining of the bone surfaces in the study group and increased mean area percent of formed bone. Radiographic bone density changes were found to be statistically significant between the two studied groups. (P2= <0.001).

**Conclusion**

The use of autogenous bone graft with hyaluronic acid appears to be **more efficient in osteoconduction** when compared with autogenous bone graft alone and could be a promising strategy for preservation of alveolar sockets.

Zhu X, von Werdt L, Zappalà G, Sculean A, Eick S, Stähli A. 'In vitro activity of hyaluronic acid and human serum on periodontal biofilm and periodontal ligament fibroblasts'. Clin Oral Investig. 2023 Sep;27(9):5021-5029. doi: 10.1007/s00784-023-05121-z. Epub 2023 Jun 28. PMCID: PMC10492760. PMID: 37380794.


**Objectives**
A beneficial effect of cross-linked hyaluronic acid (cHA) on periodontal wound healing and regeneration has recently been demonstrated. The present in vitro study was designed to obtain deeper knowledge on the effect of cHA when applied in the gingival sulcus (serum-rich environment) during non-surgical periodontal therapy.

**Materials and methods**
The influence of cHA, human serum (HS), and cHA/HS on (i) a 12-species biofilm formation, (ii) the adhesion of periodontal ligament fibroblasts (PDLF) to dentine surface, (iii) the expression and secretion of interleukin-8, and (iv) the expression of receptors of HA in PDLF and gingival fibroblasts (GF) were evaluated.

**Results**
At 4 h of biofilm formation, cHA and HS in combination (cHA/HS) slightly decreased the colony-forming unit counts in biofilm whereas the metabolic activity of biofilm was reduced in all test groups (cHA, HS, cHA/HS) vs. control. At 24 h, the quantity of biofilm was reduced in all test groups vs. untreated control. The test substances did not affect adhesion of PDLF to dentin. HS increased the expression of IL-8 by PDLF and GF which was partially downregulated by cHA. HS and/or cHA promoted the expression of the HA receptor RHAMM in GF but not in PDLF.

**Conclusions**
In summary, the present data indicate that serum neither negatively affect the activity of cHA against periodontal biofilm nor had any unwanted influence on the activity of PDLF.

**Clinical relevance**
**These findings lend additional support for the positive effects of cHA on cells involved in periodontal wound healing, thus pointing to its potential use in non-surgical periodontal therapy**.

# Relevant Studies on Hyaluronic Acid

Akyildiz S, Soluk-Tekkesin M, Keskin-Yalcin B, Unsal G, Ozel Yildiz S, Ozcan 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. PMID: 30157145.

**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.

Alcantara CEP et al. 'Hyaluronic acid accelerates bone repair in human dental sockets: a randomized triple-blind clinical trial'. Braz Oral Res. 2018:32:e84. doi: 10.1590/1807-3107bor-2018.vol32.0084. Epub 2018 Sep 13. PMID: 30231173.

**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.

Aslan M, Simsek G, Dayi E. 'The Effect of Hyaluronic Acid-supplemented Bone Graft in Bone Healing: Experimental Study in Rabbits'. J Biomater Appl. 2006 Jan;20(3):209-20. doi: 10.1177/0885328206051047. PMID: 16364962.

**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.

Aya KL, Stern R. 'Hyaluronan in wound healing: rediscovering a major player'. Wound Repair Regen. 2014 Sep-Oct;22(5):579-93. doi: 10.1111/wrr.12214. PMID: 25039417.

**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.

Bassiouny G. 'Bioinspired Approach for Dental Implant Functionalization: An Experimental Study Evaluating the Effect of Hyaluronate as Bioactive Implant Coating'. Journal of American Science 2013;9(11):187-192. ISSN: 1545-1003.

**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**.

Bayoumi A, Nadershah M, Albandar A, Alsulaimani B, Sankour I, et al. (2018). 'The Effect of Cross-Linked Hyaluronic Acid in Surgical Extraction of Impacted Mandibular Third Molars'. Int J Dent Oral Health 4(2): dx.doi.org/10.16966/2378-7090.254.


**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.

**Method and Materials**

This randomized, double-blinded, split-mouth 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 2$^{nd}$, 4$^{th}$ and 7$^{th}$ days after the surgery.

**Results**

The scores for facial swelling, pain and trismus were the highest on the 2$^{nd}$ postoperative day and decreased gradually on the 4$^{th}$ to the 7$^{th}$ 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.

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. PMID: 23444204.


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

**Method and materials**
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.

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 Oral Investig. 2019 Sep;23(9):3423-3435. doi: 10.1007/s00784-019-03012-w. Epub 2019 Jul 23. PMID: 31338632.

**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 methods**
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.

**Conclusions**
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**.

Elkarargy A. 'Alveolar Sockets Preservation Using Hydroxyapatite / Beta tricalcium Phosphate with Hyaluronic Acid (Histomorphometric study)'. Journal of American Science 2013;9(1):556-563. ISSN: 1545-1003.

**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.

Fawzi KM, Dahaba MA, Aboul-Ela S, Darhous MS. 'Local application of hyaluronan gel in conjunction with periodontal surgery: a randomized controlled trial'. Clin Oral Investig. 2012 Aug;16(4):1229-36. doi: 10.1007/s00784-011-0630-z. Epub 2011 Oct 20. PMID: 22012469.

**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.

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. PMID: 28834247.

**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).

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 Jul;87(7):790-5. doi: 10.1902/jop.2016.150707. Epub 2016 Mar 18. PMID: 26991484.

**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 remodeling phases. The objective of this study is to evaluate the effect of hyaluronic acid (HA) on healing of infected sockets.

**Methods**
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**.

King SR, Hickerson WL, Proctor KG (1991). 'Beneficial actions of exogenous hyaluronic acid on wound healing'. Surgery. 1991 Jan;109(1):76-84. PMID: 1984639.

**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**.

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. PMCID: PMC7103456. PMID: 32040462.

**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.

**Material and methods**
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 $5^{th}$, $10^{th}$, $15^{th}$, $20^{th}$, $25^{th}$ 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.

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

Mendes RM et al. 'Sodium hyaluronate accelerates the healing process in tooth sockets of rat'. Arch Oral Biol. 2008 Dec;53(12):1155-62. doi: 10.1016/j.archoralbio.2008.07.001. Epub 2008 Aug 9. PMID: 18692778.

**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.

Pirnazar P, Wolinsky L, Nachnani S, Haake S, Pilloni A, Bernard GW. 'Bacteriostatic effects of hyaluronic acid'. J Periodontol. 1999 Apr;70(4):370-4. doi: 10.1902/jop.1999.70.4.370. PMID: 10328647.

**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. 1995 Jan;16(1):9-15. doi: 10.1016/s8756-3282(94)00001-8. PMID: 7742090.

**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 remodeling 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.

Stiller M. et al. 'Performance of β-tricalcium phosphate granules and putty, bone grafting materials after bilateral sinus floor augmentation in humans'. Biomaterials. 2014 Mar;35(10):3154-63. doi: 10.1016/j.biomaterials.2013.12.068. Epub 2014 Jan 16. PMID: 24439419.


**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.

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 Jan;89(1):36-45. doi: 10.1902/jop.2017.170105. PMID: 28914592.

**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.

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

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

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