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PERIODONTAL WOUND HEALING WITH HYALURONIC ACID ALONE OR IN CONJUNCTION WITH BOVINE BONE GRAFT IN THE TREATMENT OF CLASS II FURCATION DEFECTS: A HISTOLOGIC AND HISTOMORPHOMETRIC STUDY

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ABSTRACT

Aim: The goal of the current study was to evaluate the periodontal wound healing potential of hyaluronic acid alone or in conjunction with bovine bone graft in the treatment of canine class II furcation defects.

Materials and Methods: In beagle dogs, class II furcation defects were surgically made in the mandibular premolars. Both open flap debridement (OFD) and hyaluronic acid (HA) were used to address the deficiencies in the HA group and OFD plus hyaluronic acid (HA) and bovine bone graft (BG) in the HA/BG group. The animals were euthanized at 12 weeks to allow for histological analysis.

Results: The newly formed bone height in the HA group was (3.94 ± 0.28 mm) and in HA/BG (4.19 ± 0.37mm), the newly formed bone percentage in the HA group was (73.75 ±6.28) and in HA/BG group was (76.26 ±6.49) and the thickness of the newly formed bone trabeculae in HA group was (0.38 ±0.04) and in HA/BG was (0.47 ±0.06). Statistical analysis showed no significant differences in any of the histomorphometric measures between the HA and HA/BG at ($p < 0.05$) except for the thickness of the newly formed bone trabeculae that significantly increased in HA/BG group in comparison to HA group ($p = 0.013$).

Conclusions: Within their limitations, the findings of the current study imply that using HA alone or in conjunction with BG promotes periodontal wound healing in surgically induced class II furcation defects.

KEYWORDS bone graft, furcation defects, hyaluronic acid, periodontal wound healing

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INTRODUCTION

Periodontitis is the most prevalent non-transmissible chronic inflammatory disease of humans. It is initiated by dysbiotic dental plaque biofilms.(1) It is a serious issue for public health due to its role in edentulism and masticatory dysfunction (2) It is distinguished by the progressive breakdown of the tooth-supporting structure, which displays as clinical attachment loss (CAL), radiographically identified alveolar bone loss, the formation of periodontal pocketing, and gingival bleeding.(3)

Furcation involvement is the progression of periodontitis into the furcation area of multirooted teeth (4) Predictable treatment outcome of furcation involvement remains one of the most challenging issues for the clinician. Various modalities for its management have been tried including non-surgical periodontal treatment with or without systemic or local antimicrobial agents (5, 6) Laser and photodynamic therapy have also been reported (6) Moreover surgical procedures, including open flap debridement, resective and regenerative approaches are also tried (7)

Taking into consideration the complex anatomical characteristics, including horizontal and vertical bone loss and the existence of multiple roots; furcation defects are difficult to access for optimal instrumentation. In addition, the long-term prospect of a tooth that has undergone resective surgery is debatable. (8) Moreover, it is challenging to create a biologic environment to support periodontal regeneration in the areas of furcation because of the large avascular root surfaces, horizontal and vertical attachment loss, and bone loss. (9, 10, 11)

One of the optimum goals of periodontal therapy is periodontal regeneration. It can modify the prognosis of questionable teeth to one of sustained health (12, 13, 14). Bone replacements, barrier membranes, and other biomaterials have all been employed alone or in combination for periodontal regeneration. A significant degree of patient satisfaction has been observed along with successful clinical, radiological (15-18) and histological results (19, 20)

Despite these positive outcomes, numerous studies have found inconsistencies in the results of various periodontal regenerative techniques, which could be attributable to many factors such as the diverse of the biomaterials' composition utilized, the combinations employed (21,22), and post-operative issues (23,24). The most often employed materials in this context are bone substitutes either alone or in combination with membranes. Autogenous bone was regarded to be the ideal bone transplant material for regeneration because it fulfills both osteogenicity, osteoinductivity and, osteoconductivity (25) Although being the gold standard, its use is limited by the donor-site morbidity. Furthermore limitations in terms of the amount of collected bone, inevitable bone resorption, and the larger surgical area needed to harvest autogenous bone. (26) In an attempts to overcome these issues, allogenic, and alloplastic bone substitutes have been used, but faced limitations like antigenicity, high cost, prolonged healing time, and limited osteoinductive capability. (27)

Barrier membranes, on the other hand, were introduced to achieve the goal of guided tissue regeneration through facilitating the replenishment of the formerly infected root surface with selective progenitor cells while hindering both the gingival epithelium and connective tissue from reaching the root surface. (28, 29) Unfortunately, involves particular issues such as a more intrusive flap, extra surgery to remove the membrane (for non-resorbable ones), and the possibility of membrane exposure, which may jeopardizes the results. (30)

As a consequence, new biologics with great biocompatibility, convenience of use, and reduced upfront expenses are needed to improve periodontal regeneration and boost clinical outcome. (31)

The incorporation of biomimetic substances like enamel matrix derivatives, bone morphogenetic proteins, platelet rich fibrin, and hyaluronic acid opens up new avenues for improved periodontal therapy outcomes. (32-35).

Hyaluronic acid is a glycosaminoglycan biopolymer. It is a significant natural component of the extracellular matrix in a variety of tissues, including the, joints, skin, eyes, and periodontal tissues. (36, 37)

It is frequently discovered at almost all phases of the wound healing due to its CD44 receptor, which is expressed on the cell membrane of nearly every human cell. (38) It is in charge of attracting fibroblasts from surrounding tissue to the wound site. (39) Moreover it is also an important cell behavior regulator, influencing migration, proliferation, and stem cell differentiation. (40)

Hyaluronic acid also possesses antioxidative (41), anti-inflammatory, anti-edematous (42) and antibacterial properties.(43) Furthermore, other studies demonstrated that Hyaluronic acid significantly stimulates clot formation (44) angiogenesis (45, 37) and increases osteogenesis (46, 37)

During angiogenesis, endothelial cell behavior is significantly influenced by hyaluronic acid (37, 45). Low molecular weight hyaluronic acid enhances vascular endothelial cell proliferation and migration, and the expression of signaling molecules for cellular adhesion (47)

Hyaluronic acid is regarded as an appealing building component for use in a variety of medical sectors. Its varied applications have been intensively researched in recent years and continue to do so.

In the last few years, the topical application of hyaluronic acid has grown to include the entire spectrum of reliable regenerative materials due to its favorable benefits in clinical attachment level (CAL) gain and pocket probing depth (PPD) reduction when used in both non-surgical and surgical periodontal therapy. (36)

The aim of this study was to histologically evaluate the efficacy of hyaluronic acid, alone or in conjunction with bovine bone graft, on periodontal wound healing in class II furcation defects in dogs.

MATERIALS AND METHODS

Study sample and setting

Animals

In the current research, five healthy male beagle dogs aged 18-24 months and weighing 10- 18 kg were utilized. They were taken from the City of Scientific Research and Technological Application's animal house in Burj AL Arab, Alexandria, Egypt. Animals were kept in the same environmental and dietary circumstances at the Faculty of Medicine, Alexandria University, Egypt. All animal treatments adhere to the National Research Council Guidelines for the Care and Use of Laboratory Animals (48). The histological and histomorphometric analysis were carried out in the Department of Oral Biology, Faculty of Dentistry, Alexandria University. The research protocol was approved by the institutional experimentation and Animal Ethical Committee of Alexandria University, Institutional Review Board (IRB: 00010556-IORG0008839)

Sample size

The sample size was calculated using a 5% alpha error and an 80% study power. The mean and standard deviation (SD) of newly formed bone was 9.5% (3.63) and 15.2% (3.18) for Enamel Matrix derivative (EMD) as biological factor with bovine bone and EMD alone, respectively. (49) We used this study for sample size calculation as they used the same protocol as ours and because both EMD and HA are matched in their results as being nearly two similar biologics in periodontal regeneration. (5, 19, 50) Based on difference between dependent means, a sample of 8 sites per group was required with an effect size of 1.181. This was increased to 10 sites per group in order to make up for process errors. G*Power was used to compute the sample. 3.1.9.7. (51)

Inclusion criteria:

- Dogs in good health,
- Dogs that were matched in terms of age, gender, weight, food type, and living circumstances.

Exclusion criteria:

- Dogs that have already participated in an experimental research.
- Dogs with obvious illnesses or wounds.

Surgical protocol

The animals were anaesthetized with an intravenous dose of sodium thiopental (13 mg/kg) (Sandoz GmbH Biochemiestraße, Austria). Antibiotics (Ampicillin, Eipico, Tenth of Ramadan City, Egypt) were intramuscularly given prior to the surgical operations.

Experiments were carried out on the mandibular third and fourth premolars (P3 and P4) bilaterally (four locations per dog) (Figure1a). Class II acute furcation defects had been created surgically using round burs and bone chisels under irrigation with saline after sulcular incisions and elevation of the mucoperiosteal flap. The created furcation deficiencies were measured 5.0 mm in the mesio-distal and 5.0 mm in the occluso-apical direction using a periodontal probe. Bone was partially removed from the buccal sides between the mesial and distal roots. Gracey curettes were used to plan the exposed root surfaces. (Figure1b). To identify the exact extension of the furcation defects and to permit for efficient histomorphometric analysis, reference notches were created on the crown surface and on the root surface (at the base of the defects) using a #1 round bur. The following interventions were given to the 20 bilateral furcation defects: hyaluronic acid gel (HA)(GENGIGEL®, Ricerfarma srl, Milano, Italy)) alone in 3rd premolar (HA group); HA + compact bovine bone graft (BG) (The Graft, Purgo Biologics Inc., Korea) in 4th premolar (HA/BG groupl). In the HA group, the HA (0.2 ml/defect) was placed to the root surfaces, and the defects were filled to the extent of the adjacent alveolar crest (Figure1b). In the HA/BG group, the bone graft was entirely soaked with HA (0.2 ml/mg BG). The combination was then gently pressed into the defects. (Figure 1c). To ensure complete tension-free covering of the furcation defects and the biomaterials, a periosteal releasing incision was performed to allow coronal displacement of the flap, followed by suturing slightly coronal to the CEJ (Figure 1d). After a couple of weeks, the sutures were removed.

Post-surgical protocol

For the first two weeks after surgery, the animals were provided with a soft food. The antibiotic ampicillin (Eipico, the Tenth of Ramadan City, Egypt) was administered intramuscularly on the first day and then combined with the dog's food for the next seven days. Intramuscular (IM) injections of non-steroidal anti-inflammatory (Meloxicam DELTA PHARMA Factory Industrial Zone B4, the Tenth of Ramadan City, Egypt (0.09 mg/lb) were also administered for the next two days. For 12 weeks following surgery, plaque control was maintained by regular cleaning of the oral cavity with 2% chlorhexidine HCL (Hexitol mouth wash, The Arab Drug Company (ADCO) - ARE).

Histological procedure

Twelve weeks following surgery (31), dogs were sacrificed using thiopental sodium overdose (intravenously). Surgical sites were excised and fixed in 10% neutral buffered formalin. The tissue samples were thereafter decalcified in 10% trichloroacetic acid and histologically handled to yield 5 micron thick slices that were stained for histological examination with:

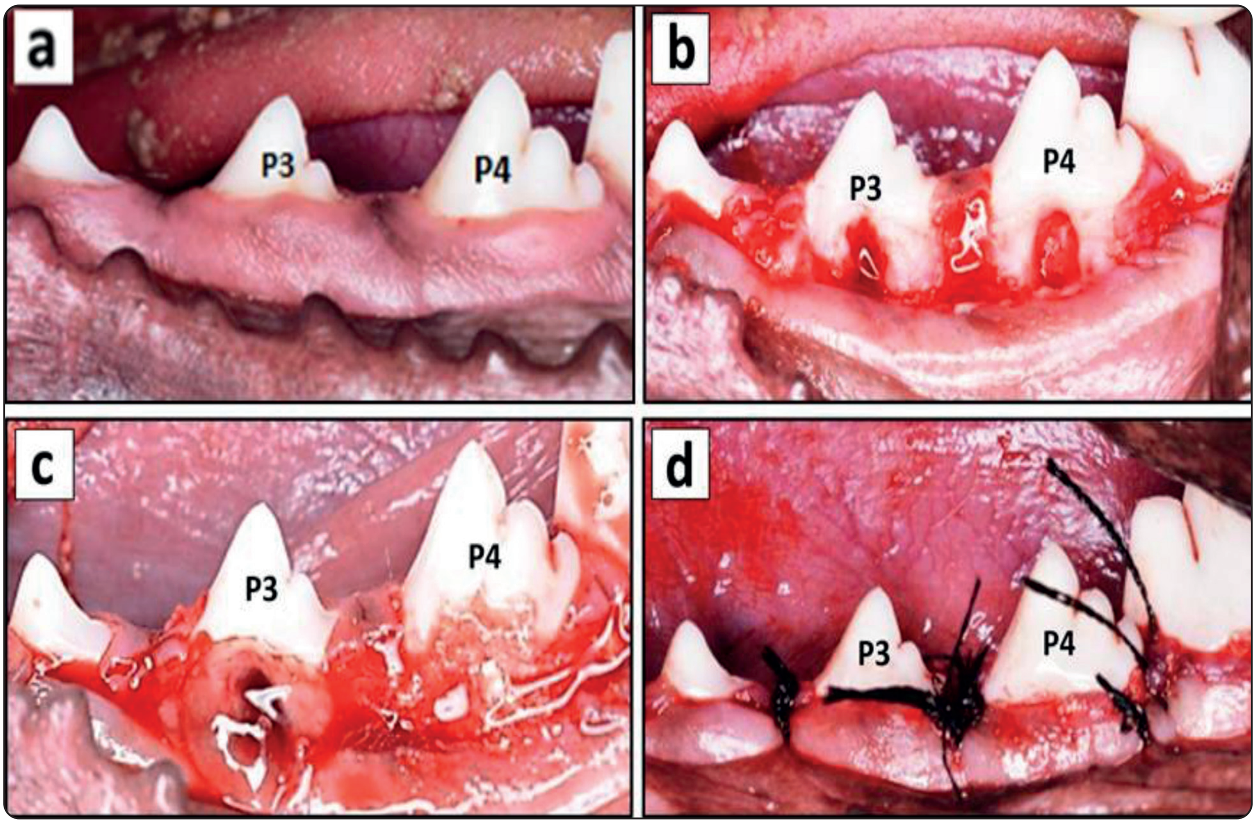


Fig. (1): displays the surgical approach. (a) Before undergoing regenerative surgery. (b) Surgically created class II furcation defects in P3 and P4 (c) The defects received (HA) gel alone in (P3) and (HA/BG) in P4 (d) Flap repositioning and suturing

a) H&E (Hematoxylin and Eosin).

b) Gomori Trichrome stain

Trichrome stain is basically used to demonstrate connective tissue elements,principally collagen fibers,which is stained green while basophilic structures and nuclei stained blue to vilot.

Quantitative histomorphometry analysis

Image J software (Image J version 1.46r; NIH, Bethesda, [MD], USA, https:// imagej. nih. gov/ ij/ downl oad. html) (52) was used to analyse light microscopic photomicrographs at standardised magnification. Three factors were investigated:

1. New formed bone height in the interradicular area, measured in millimetres (mm). This is the distance with a standardised magnification of 40x from the most coronal point of the formed bone to a line crossing between the two notches on the mesial and distal roots at the most apical end.
2. The mean percentage of newly formed bone surface area with a standardised magnification of 100x.
3. The thickness of newly formed bone trabeculae in mm with a standardised magnification of 100x,

Steps of measuring the thickness of the formed bone trabeculae

1. Measurements of the thickness of bone trabeculae were done on the same rectangles used to measure the bone surface area.
2. A straight line parallel to the apical line of the defect was drawn connecting the two closest marrow spaces at the same plane. A Similar line

was drawn connecting the two farthest marrow spaces in one plane.

3. The lengths of these two lines were measured by choosing measure from analyze then recorded
4. The average of the two values was calculated to record a value of mean thickness of bone trabeclae from each photograph

Statistical analysis

The Shapiro Wilk test and Q-Q plots were used to determine normality. All variables were regularly distributed, provided with mean and standard deviation, and compared using the paired t test. The significance threshold was set at 0.05. The data was analysed with IBM SPSS version 23.

RESULTS

Clinical findings

All the studied animals were adequately tolerated the surgical procedures. The post-surgical healing went well. Throughout the whole experiment, no significant inflammation, edema, or flap dehiscence were noticed.

Histological results

Hyaluronic acid group

The interradicular defects contained considerable amount of periodontal tissues, including bone, periodontal ligament and cementum. The formed bone occupied wide areas of the defects including the central zones. The formed bone included thick intercommunicating trabeculae, areas of compact variety and some regions of immature bone that contained many trapped osteocytes. This variety of bone was localized at most coronal regions in some defects. Small fibrous tissue sheets were seen among the formed bone and contained small blood vessels (figures 2 a&b)

In section taken from variable depths of the defects a noticeable degree of tissue regeneration was noted. The bone border adjacent to the forming PDL exhibited histological features of remodeling with small concavities of resorption superimposed with new fiber attachment (figure 2c). Areas of active bone formation with voluminous osteoblasts were seen adjacent to definite areas of osteoid (figure 2d). Also newly formed layers of cementum were traced on the root borders facing the defects. These layers accommodated the insertion of regenerating PDL fibers among which cementoblasts were seen arranged in more than one raw. These fibers appeared in streams directed to the opposing interface of the regenerating bone and contained rich irregularly oriented cellular components. Among these fiber streams grouping, it was hard to follow definite individual fiber bundle course ((figures 2 e&f)

In other words, it can be mentioned that the defects exhibited considerable amounts of PDL fiber formation and aggregation but slightly lacking the proper final organization.

In trichrome stained sections, two important observations were noted; one observation related to fiber aggregation in specific directivity for mapping other trabeculae or adding more body to the already formed trabeculae (figure 2g). The other observation related to well-developed osteoblasts seen at the periphery of the growing and regenerating trabeculae with an appearance of being involved in osteoid formation at these sites (figure 2h)

Hyaluronic acid + bone graft group

The histological picture of this group revealed more organizing pattern of tissue regeneration than that seen in Hyaluronic acid group alone with outstanding biological features of regenerating tissues. Greater amount of formed bone occupied most of the defects' surface area. The formed bone consisted partly of compact and partly of cancellous varieties. The latter exhibited outstanding pattern of thick and intercommunicating trabeculae, while the compact variety prevailed in the sections obtained from most of the examined defects. (figure 3 a&b)

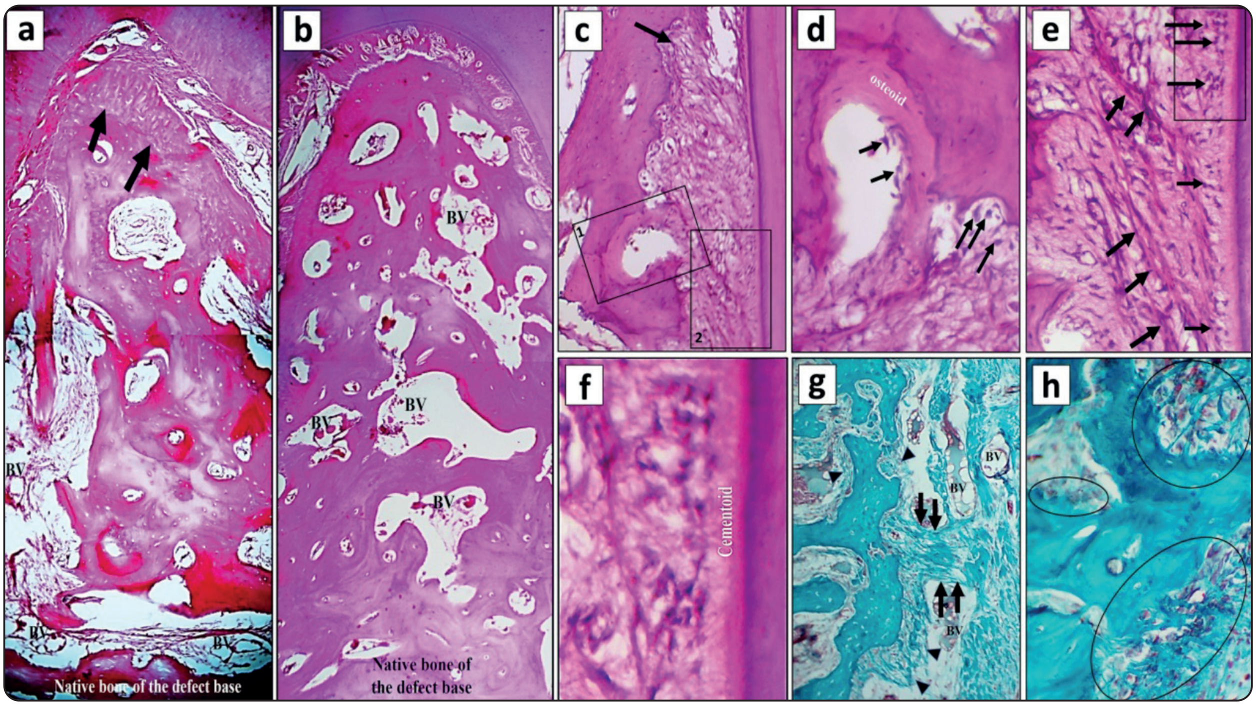


Fig. (2): Hyaluronic acid (HA) group, (a-f: H&E stain). (a&b): Compound images showing the organization of the regenerating tissues in the healed defects. Notice the intercommunicating thick trabeculae of the formed bone and some of the compact variety together with small areas of newly formed bone (arrows). Many blood vessels (BV) are seen in the fibrous tissue among the formed bone, original magnification X: 40 (c): Showing small bony depressions of recent resorption with the formed fiber re-attachment (arrow), original magnification X: 100. (d): Showing higher magnification of inset 1 in the previous figure revealing continuation of bone formation at an active area of remodeling and osteoid formation. Osteoblasts are seen adjacent to the formed osteoid and at the resorption pays (arrows), X: 400. (e): Showing higher magnification of inset 2 in (figure 2c) revealing a regular layer of cementoid formation close to the root surface and the adjoining cementoblasts (thin arrows). Note course of streams of fiber grouping from cementum to the regenerating alveolar bone (thick arrows) and the irregularly distributed fibroblasts, X: 400 (f): Showing magnified photo of the boxed part in the previous figure revealing voluminous cementoblasts adjacent to a layer of formed cementoid. original magnification, X: 400. (g&h: Trichrome stain), (g): Showing organization of the regenerating PDLfibers in specific directivity for mapping additional bone segments (arrows) or adding more body to an existing bone segment (arrow heads). Note the rich blood supply (BV) among the fibrous tissue, X: 100. (h): Showing voluminous osteoblasts at the periphery of the regenerating and growing trabeculae in the defect within the developing fibers of osteoid (circles), X: 400

PDL fiber formation and attachment between this bone and the forming thick cementum were seen spanning the distance between the two tissues (figure 3c), also broad lines of union between the successive formed layers of the regenerating bone in the defects were evident (figure 3d). At some regions of the defects small segments of bone formation were seen connected by a background of fibrous textured stroma rich in blood supply. This appearance is thought to reflect an inducible biological effect shared by more than one factor (figure 3e).

The appearance of regenerating PDL fibers exhibited variability in sections taken at different levels of cutting. In their course from cementum to bone the fibers appeared thin, irregularly and densely interlacing especially at the PDL center, then formed definitive insertion in both tissues. In association with these fibers, fibroblast cells appeared small,

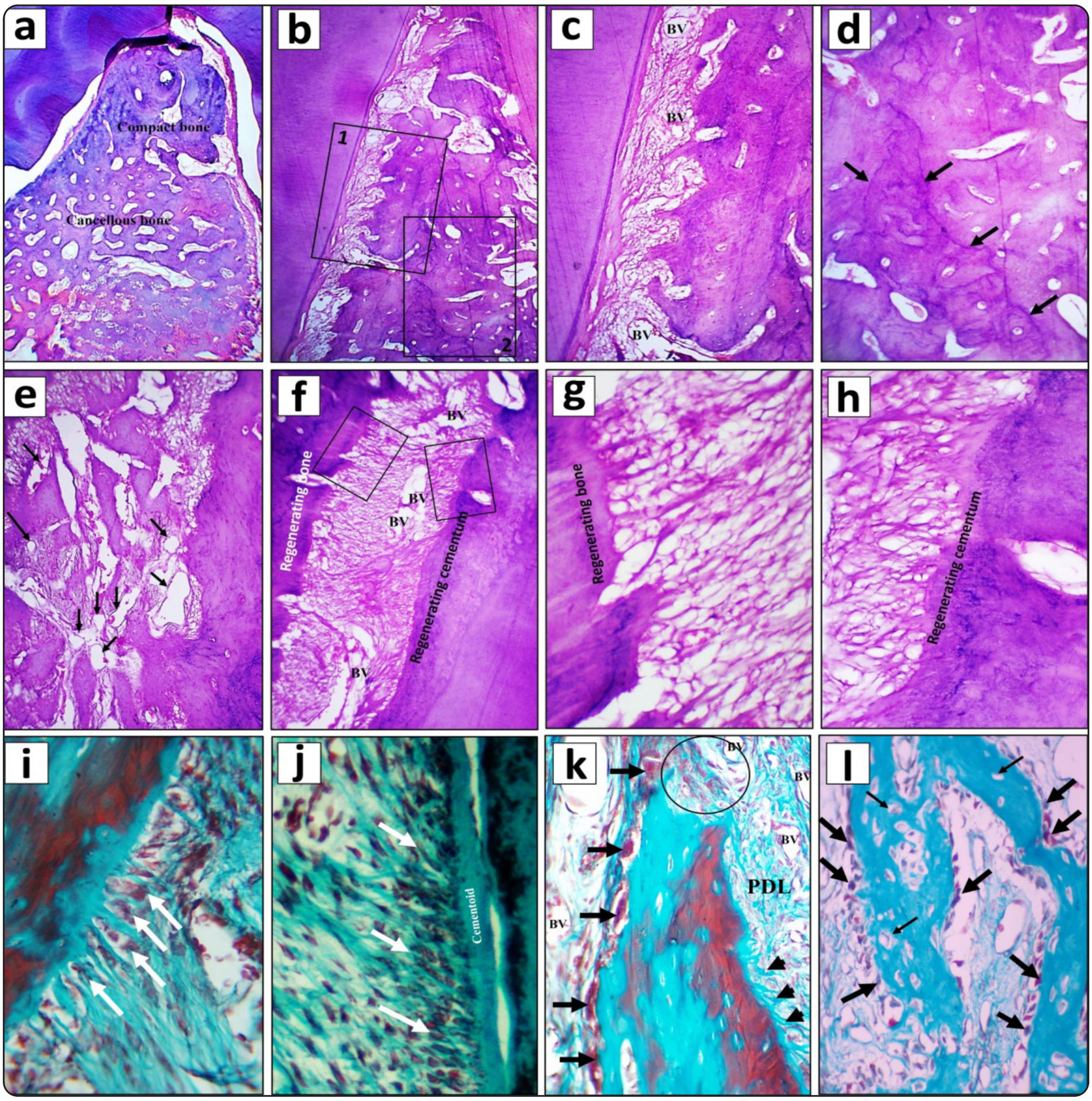


Fig. (3): Hyaluronic acid and bone graft (HA/BG) group, (a-h: H&E stain). (a): Showing complete filling of the defect with partly cancellous and partly with compact varieties of the regenerating bone. Intercommunicating trabecular pattern is seen. X: 40 (b): Showing formation of considerable amount of regenerating bone in the healing defect with predominance of the compact variety, X: 40.(c): Showing higher magnification of inset 1 in figure 3b revealing PDL fiber formation and attachment between the developing cementum and bone. Many blood vessels (BV) are seen among the regenerating periodontal tissues. Note the thickness of the formed cementum adjacent to the root surface, X:100 .(d): Higher magnification of inset 2 in figure 3b revealing lines of union between the successive layers of the formed bone (arrows) and the variable degrees of their maturation, X:100. (e): Showing formation of small bone segments of variable sizes on a fibrous textured stroma rich in blood supply (thin arrows) and surrounded by a large segment exhibiting different levels of bone maturation at its periphery, X: 100. (f): Reveals formation of irregular densely interlacing PDL fibers spanning the distance between the forming bone and cementum and accommodating many blood vessels (BV). X: 100. (g&h): Showing higher magnification of the two insets in (f) revealing fiber interlacing and definite insertion in both tissues. Small, flattened and even inconspicuous fibroblasts associate the thin and irregularly forming PDL fibers, (g&h) X:400. (i-l: Trichrome stain), (i&j): Reveal normal organization, course, and density of regenerating PDL fibers and their insertion in bone (i) and in cementum (j) respectively while separated by noticeable density of voluminous osteoblasts and cemntoblasts (arrows). (i&j X:400). (k): Showing osteoclast cells (arrows) on one side of a bone segment facing the periodontal ligament with neighboring osteoblasts exhibiting formative activity of bone collagen fibers (circle). Note the rich blood supply in the regenerating tissue (BV) and fiber attachment on the opposite side of the regenerating bone (arrow heads), X: 400 .(l): Showing many osteoblasts on the border of one of the trabeculae in the regenerating defects (arrows). Large spaces of osteocytes' lacunae are seen in these trabeculae (thin arrows), Note the delicate fibrous tissue stroma surrounding the trabeculae and compare that appearance to that seen in figure (3e), X: 400.

flattened and even inconspicuous (figure 3 f-h). Another appearance involved highly organized fiber bundles that exhibited typical course from bone to cementum where their insertion in these tissues constituted Sharpey's fibers. In this situation normal density and orientation of fibroblast cells were noted. This appearance was traced in trichrome stained sections which also revealed noticeable density of osteoblasts and cementoblasts adjacent to the regenerating bone and cementum respectively while separated by the inserting fibers (figure 3 i&j)

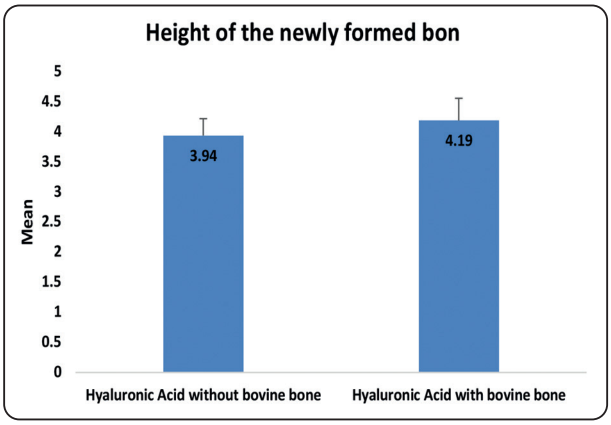
Also, in trichrome stained sections; the regenerative features in the formed periodontium were clearly revealed. Persistent remodeling and maturation figures were traced, osteoclasts were seen in Howships lacunae on the border of some bone segments facing the PDL while adjacent to them aggregates of osteoblast cells were seen exhibiting formative activity of bone collagen fiber formation (figure 3k). In addition to osteoclast activity, many osteoblasts were traced on the border of few trabeculae which exhibited high density of large spaces of osteocytes in an appearance equivalent to that of the small bone segments seen in figure (3e) but here it is providing better details of the stroma due to the specific trichrome stain, (figure 3 l)

Histomorphometric results:

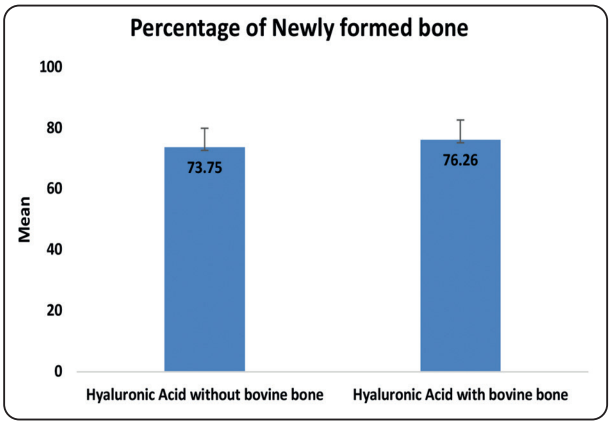
The Quantitative histomorphometric results of the current study revealed that the combination of hyaluronic acid and bovine bone graft (HA+BG) can enhance periodontal regeneration in class II furcation defects when compared to hyaluronic acid (HA) alone. These findings were evidenced by the increase in the height of newly formed bone (4.19 ±0.37 mm) for (HA+BG) versus (3.94 ±0.28 mm) for (HA) alone, however this increase was not statistically significant (p value=0.15). Moreover, the increase in the percentage of the newly formed bone was (76.26 ±6.49) for (HA+BG) versus (73.75 ±6.28) for (HA) alone, also, this increase was not statistically significant (p value=0.509). Furthermore, the thickness of the formed bone trabeculae was (0.47 ±0.06) for (HA+BG) versus. (0.38 ±0.04) for (HA) alone. The combination of hyaluronic acid and bovine bone (HA+BG) have yielded a statistically significant increase in the thickness of the newly formed bone trabeculae compared to the sites treated with hyaluronic acid (HA) alone (p value=0.013* table(1).

TABLE (1): Comparison of bone height, percentage of newly formed bone and thickness of bone trabeculae between Hyaluronic Acid alone or in combination with bovine bone

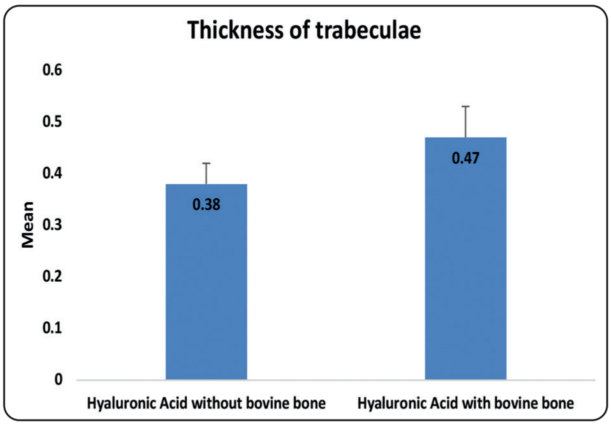
	Hyaluronic Acid without bovine bone (n=10)	**Hyaluronic Acid with bovine bone (n=10)**	Test (P value)
	Mean (SD)		
Height of the newly formed bone	3.94 (0.28)	4.19 (0.37)	1.534 (0.159)
Percentage of Newly formed bone	73.75 (6.28)	76.26 (6.49)	0.688 (0.509)
Thickness of bone trabeculae	0.38 (0.04)	0.47 (0.06)	3.096 **(0.013*)**



Graph (1) The mean of the height of the newly formed bon between Hyaluronic Acid alone or in combination with bovine bone



Graph (2) The mean of the percentage of the newly formed bon between Hyaluronic Acid alone or in combination with bovine bone



Graph (3) The mean of the Thickness of trabecula of the newly formed bon between Hyaluronic Acid alone or in combination with bovine bone

DISCUSSION

Periodontal regeneration is the pinnacle goal of periodontal treatment. Regenerative interventions have undergone numerous advances in terms of both methodology and biomaterials during the previous several decades. (53) Various biologics, either alone or in combination with bone graft, have sparked considerable interest in regenerative periodontics in recent decades (54-56). Clinical trials have demonstrated their effectiveness in attaining significant gain of CAL and reduction in PPD. (50,57,58) HA is currently gaining attraction, with various clinical studies being carried out to evaluate its effectiveness in periodontal regeneration (59,60). The goal of this study was to histologically and histomorphometrically evaluate the effectiveness of hyaluronic acid (HA) alone or in combination with bovine bone on periodontal wound healing in class II furcation defects in dogs.

The results of the current experimental study revealed that the combination of HA and bovine bone graft (BG) can yield substantial periodontal regenerative potential in class II furcation defects when compared to hyaluronic acid (HA) alone; evidenced by the increase in the height of newly formed bone (4.19 ±0.37 mm) versus. (3.94 ±0.28 mm), percentage of newly formed bone (76.26 ±6.49) versus. (73.75 ±6.28) and the thickness of the formed bone trabeculae (0.47 ±0.06) versus. (0.38 ±0.04) for the combination (HA+BG) versus (HA) alone respectively. Despite not statistically significant regarding the height of the newly formed bone (p value=0.15) and percentage of newly formed bone (p value=0.509), the combination of hyaluronic acid and bovine bone (HA+BG) have yielded a statistically significant increase in the thickness of the newly formed bone trabeculae compared to the sites treated with hyaluronic acid (HA) alone (p value=0.013* table(1).

This is thought to reveal more synthetic activity of osteoblasts in (HA/BG) group as evidenced also by histological findings of the sections stained with H&E where the bone graft proved to have provided an excellent osteoconductive effect that had increased the recruitment of the cells to the defect leading to more bulk of bone formation that was exhibited in the increased trabecular thickness.

On the other hand the sections stained with Trichrom stain in both groups were also revealed the presence of regenerating periodontal ligament fibers course while inserting in the newly formed cementum and bone while separated by the distinctly appearing cells (fig2g,2h,3k,3l)

The work by Shirakata et al (2022)(31), who histologically investigated the effectiveness of cross linked hyaluronic acid (HA) alone or combined with collagen matrix (CM) on periodontal regeneration of class III furcation defects in dogs, is of special relevance to our findings. They found that the surface area of the regenerated bone was greater in the (HA) (4.04 ± 1.51) and HA/CM (4.32 ±1.14 mm^2) groups compared to the OFD (3.25± 0.81 mm^2) and CM (3.31 ±2.26 mm^2) groups. Furthermore, they assessed the production of new connective tissue attachment and discovered that the HA (6.25 ±1.45 mm) and HA/CM (6.40 ±1.35 mm) groups produced statistically significant ($p < .05$) formation of new connective tissue attachment than the OFD (1.47±0.85 mm). There results also revealed the absence of any significant differences between the (HA) and HA/CM groups in any of the histomorphometric parameters.

At the same context, our results are comparable to those of Shirakata et al as regards their preclinical study in dogs in 2021(61) .They investigated the effect of cross-linked hyaluronic acid (HA) alone or in conjunction with collagen matrix (HA/CM) on periodontal wound healing of two-walls intrabony defects following reconstructive surgery. They reported that the newly formed trabecular bone within 5 × 5-mm defect was (3.91± 3.10) for HA sites and (4.74 ± 1.76) for HA/CM compared with (3.01 ± 1.75) for OFD sites. Also they have found that sites treated with HA alone (2.43 1.25± mm) and HA/CM (2.60 0.99± mm) revealed statistically significant ($P < .05$) formation of new attachment compared with the sites managed by OFD only (0.55 0.99 ± mm). They concluded that, the HA/CM group showed the greatest level of regenerated tissues, although no statistically significant differences in any of the histomorphometric parameters were obtained between the HA and HA/CM groups. This can be interpreted by the fact that the actual regenerative features observed in both groups can be mainly attributed to the effect of HA on periodontal cells since it is the only biological material used in both groups.

On the other hand, the findings of clinical trials done to assess the efficacy of hyaluronic acid alone or in combination with bone graft on wound healing of intrabony defects have yielded positive outcomes; Ahmed M.El-Bana et al 2020 (62) studied the clinical and radiographic effects of hyaluronic acid (HA) in conjunction with beta-tricalcium phosphate (TCP) in the treatment of intrabony defects in periodontitis patients. They revealed that clinical periodontal markers improved considerably following therapy in all test groups as compared to the baseline. They concluded that combination of Hyaluronic acid and (βTCP) gave good results in the treatment outcomes as regard bone level and periodontal clinical parameters.

A different approach by Božic, D.2021 (63) assessed the clinical results of a conjunction of hyaluronic acid (HA) and deproteinized bovine bone mineral in managing deep intrabony defects applying papilla preservation techniques and declared that at 6 months, there was a significant gain in CAL of 3.65 _ 1.67 mm ($p < 0.001$) with a PPD reduction of 4.54 _ 1.65 mm ($p < 0.001$). They came to the conclusion that using a combined HA and xenograft technique is a genuine novel strategy for treating intrabony defects and results in clinically relevant CAL gain and PPD declines when compared to baseline values.

Comparing the current findings to those previously discussed indicates that HA in conjunction with a bone grafting material had a synergistic impact on the regeneration of class II furcation defects as opposed to the use of HA alone. One of the possible causes of these variances is that HA, like other biologics, has a fluid nature that makes it impossible for it to have the space-creating ability required for periodontal regeneration. This could eventually cause the mucoperiosteal flap to collapse, limiting the results of regenerative surgery. Combining a bone grafting material with HA might therefore give adequate support to the flap, preventing it from collapsing into the defect, consequently stabilizing the blood clot and allowing ample space for periodontal regeneration.

Limitation

The lack of a negative control group (open flap debridement (OFD) alone and a group treated with bovine bone alone is a limitation of the current study, although shirakata et al 2022 (31) disclosed that the regenerative efficacy of HA alone or in combination with collagen membrane (CM) for the management of class III furcation defects in dogs is superior to those treated by either (CM) alone or (OFD) alone.

Conclusion &Recommendations

Within their limitations, the current findings imply that using HA alone or in conjunction with bone graft improves periodontal wound healing in surgically created class II furcation defects. As a result, it is possible that this modality will provide an innovative therapeutic approach for furcation defect challenges. To shed additional light on the therapeutic significance of this unique therapy, randomized controlled clinical trials comparing the use of HA alone or in conjunction with bone graft are warranted.

REFERENCES

1. Sanz M, Herrera D, Kebschull M, et al; On behalf of the EFP Workshop Participants and Methodological Consultants. Treatment of stage I–III periodontitis—The EFP S3 level clinical practice guideline. J Clin Periodontol. 2020;47:4–60. https://doi.org/10.1111/jcpe.13290
2. Tonetti, M. S., Jepsen, S., Jin, L., & Otomo-Corgel, J. (2017). Impact of the global burden of periodontal diseases on health, nutrition and wellbeing of mankind: A call for global action. Journal of Clinical Periodontology, 44(5), 456–462. https://doi.org/10.1111/jcpe.12732.
3. Papapanou, P. N., Sanz, M., Buduneli, N., Dietrich, T., Feres, M., Fine, D. H.,Tonetti, M. S. (2018). Periodontitis: Consensus report of workgroup2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. Journal of Clinical Periodontology, (Suppl 20), S162–S170. https://doi.org/10.1111/jcpe.12946
4. Müller HP, Eger T. Furcation diagnosis. J Clin Periodontol 1999;26:48598.
5. Pradeep AR, Priyanka N, Kalra N, Naik SB, Singh SP, Martande S. Clinical efficacy of subgingivally delivered 1.2-mg simvastatin in the treatment of individuals with Class II furcation defects: a randomized controlled clinical trial. J Periodontol. 2012;83(12):1472-9.
6. Masaeli R, Zandsalimi K, Lotfi Z, Tayebi L. Using Enamel Matrix Derivative to Improve Treatment Efficacy in Periodontal Furcation Defects. J Prosthodont. 2018;27(8):733-6.
7. Graziani F, Gennai S, Karapetsa D, Rosini S, Filice N, Gabriele M, et al. Clinical performance of access flap in the treatment of class II furcation defects. A systematic review and meta-analysis of randomized clinical trials. J Clin Periodontol. 2015;42(2):169-81.
8. Bower, R. C. (1979). Furcation morphology relative to periodontal treatment. Furcation root surface anatomy. Journal of Periodontology, 50(7), 366–374. https://doi.org/10.1902/jop.1979.50.7.366
9. Waerhaug, J. (1980). The furcation problem. Etiology, pathogenesis, diagnosis, therapy and prognosis. Journal of Clinical Periodontology, 72(2),73–95. https://doi.org/10.1111/j.1600-051x.1980.tb01951.x
10. Laugisch, O., Cosgarea, R., Nikou, G., Nikolidakis, D., Donos, N., Salvi, G. E., Stavropoulos, A., Jepsen, S., &

Sculean, A. (2019). Histologic evidence of periodontal regeneration in furcation defects: A systematic review. Clinical Oral Investigations, 23(7), 2861–2906. https://doi.org/10.1007/s00784-019-02964-3Jepsen

11. Jepsen, S., Gennai, S., Hirschfeld, J., Kalemaj, Z., Buti, J., & Graziani, F. (2020). Regenerative surgical treatment of furcation defects: A systematic review and Bayesian network meta-analysis of randomized clinical trials. Journal of Clinical Periodontology, 47(Suppl. 22), 352–374.https://doi.org/10.1111/jcpe.13238

12. Cortellini, P., Buti, J., Pini Prato, G., & Tonetti, S. (2017). Periodontal regeneration compared with access flap surgery in human intra-bony defects 20-year follow-up of a randomized clinical trial: Tooth retention, periodontitis recurrence and costs. Journal of Clinical Periodontology,44(1), 58–66.

13. Cortellini, P., Stalpers, G., Mollo, A., & Tonetti, M. S. (2020). Periodontal regeneration versus extraction and dental implant or prosthetic replacement of teeth severely compromised by attachment loss to the apex: A randomized controlled clinical trial reporting 10-year outcomes, survival analysis and mean cumulative cost of recurrence. Journal of Clinical Periodontology, 47(6), 768–776. https://doi.org/10.1111/jcpe.13289

14. Stavropoulos, A., Berti, K., Spineli, L. M., Sculean, A., Cortellini, P., &Tonetti, M. (2021). Medium- and long-term clinical benefits of periodontal regenerative/reconstructive procedures in intrabony defects: Systematic review and network meta-analysis of randomized controlled clinical studies. Journal of Clinical Periodontology, 48(3), 410 430. https://doi.org/10.1111/jcp.13409

15. Camelo, M., Nevins, M. L., Schenk, R. K., Simion, M., Rasperini, G., Lynch, S. E., & Nevins, M. (1998). Clinical, radiographic, and histologic evaluation of human periodontal defects treated with Bio-Oss and Bio-Gide. International Journal of Periodontics and Restorative Dentistry,18(4), 321–331. https://doi.org/10.1007/s00784-004-0254-7

16. Kitamura, M., Akamatsu, M., Kawanami, M., Furuichi, Y., Fujii, T., Mori, M., Kunimatsu, K., Shimauchi, H., Ogata, Y., Yamamoto, M., Nakagawa, T., Sato, S., Ito, K., Ogasawara, T., Izumi, Y., Gomi, K., Yamazaki, K., Yoshie, H., Fukuda, M., Murakami, S. (2016). Randomized placebo controlled and controlled non-inferiority phase III trials comparing trafermin, a recombinant human fibroblast growth factor 2, and enamel matrix derivative in periodontal regeneration in intrabony defects Journal of Bone and Mineral Research, 31(4), 806–814. https://doi.org/10.1002/jbmr.2738

17. Miron, R. J., Sculean, A., Cochran, D. L., Froum, S., Zucchelli, G., Nemcovsky, C., Donos, N., Lyngstadaas, S. P., Deschner, J., Dard, M., Stavropoulos, A., Zhang, Y., Trombelli, L., Kasaj, A., Shirakata, Y., Cortellini, P., Tonetti, M., Rasperini, G., Jepsen, S., & Bosshardt, D. D. (2016). Twenty years of enamel matrix derivative: The past, the present and the future. Journal of Clinical Periodontology, 43(8), 668– 683. https://doi.org/10.1111/jcpe.12546

18. Li, F., Yu, F., Xu, X., Li, C., Huang, D., Zhou, X., Ye, L., & Zheng, L. (2017). Evaluation of recombinant human FGF-2 and PDGF-BB in periodontal regeneration: A systematic review and meta-analysis. Scientific Reports, 7(1), 65. https://doi.org/10.1038/s41598-017-00113-y.

19. Sculean, A., Nikolidakis, D., Nikou, G., Ivanovic, A., Chapple, I. L., & Stavropoulos, A. (2015). Biomaterials for promoting periodontal regeneration in human intrabony defects: A systematic review. Periodontology 2000, 68(1), 182–216. https://doi.org/10.1111/prd. 12086

20. Nibali, L., Koldou, V. P., Nieri, M., Barbato, L., Pagliaro, U., & Cairo, F. (2020). Regenerative surgery versus access flap for the treatment of intrabony periodontal defects. A systematic review and meta-analysis. Journal of Clinical Periodontology, 47(Suppl. 22), 320–351. https://doi.org/10.1111/jcpe.13237

21. Tu, Y. K., Woolston, A., & Faggion, C. M., Jr. (2010). Do bone grafts or barrier membranes provide additional treatment effects for infrabony lesions treated with enamel matrix derivatives? A network metaanalysis of randomized-controlled trials. Journal of Clinical Periodontology, 37(1), 59–79. https://doi.org/10.1111/j.1600-051X.2009.01499.x

22. Lee, J. Y., Na, H. J., Kim, H. M., Lee, S. C., Lee, J. Y., Chung, C. P., ol, Y. J., & Park, Y. J. (2017). Comparative study of rhPDGF-BB plus equine-derived bone matrix versus rhPDGF-BB plus beta-TCP in the treatment of periodontal defects. International Journal of Periodontics and Restorative Dentistry, 37(6), 825–832. https://doi.org/10.11607/prd.3401

23. Sanz, M., Tonetti, M. S., Zabalegui, I., Sicilia, A., Blanco, J., Rebelo, H., Rasperini, G., Merli, M., Cortellini, P., & Suvan, J. E. (2004). Treatment of intrabony defects with enamel matrix proteins or barrier membranes: Results

from a multicenter practice-based clinical trial. Journal of Periodontology, 75(5), 726–733. https://doi.org/10.1902/jop.2004. 75.5.726

24. Yoshinuma, N., Sato, S., Fukuyama, T., Murai, M., & Ito, K. (2012). Ankylosis of nonresorbable. Hydroxyapatite graft material as a contributing factor in recurrent periodontitis. International Journal of Periodontics and Restorative Dentistry, 32(3), 331–336.
25. Schallhorn RG. Long term evaluation of osseous grafts in periodontal therapy. Int Dent J 1980;30:101-116.
26. Sakkas, A.;Wilde, F.; Heufelder, M.;Winter, K.; Schramm, A. Autogenous bone grafts in oral implantology-is it still a "gold standard"? A consecutive review of 279 patients with 456 clinical procedures. Int. J.Implant. Dent. 2017, 3, 23. [CrossRef]
27. Kim SG, Yeo HH, Kim YK. Grafting of large defects of the jaws with a particulate dentin-plaster of Paris combination. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;88:22-25. doi: 10.1016/S1079-2104(99)70188-5
28. Kannan AL, Bose BB, Muthu J, Perumalsamy R, Pushparajan S, Namasivayam A. Efficacy of combination therapy using anorganic bovine bone graft with resorbable GTR membrane vs. open flap debridement alone in the management of grade II furcation defects in mandibular molars - A comparative study. J Int Soc Prev Community Dent. 2014;4(Suppl 1):S38-43.
29. Salamanca E, Tsai CY, Pan YH, Lin YT, Huang HM, Fiveg NC, et al. In Vitro and In Vivo Study of a Novel Porcine Collagen Membrane for Guided Bone Regeneration. Materials (Basel). 2016;9
30. Francesco Tarallo, Leonardo Mancini, Luciano Pitzurra, Sergio Bizzarro, Michele Tepedino and Enrico Marchetti Use of Platelet-Rich Fibrin in the Treatment of Grade2 Furcation Defects: Systematic Review and Meta-AnalysisJ. Clin. Med. 2020, 9, 2104; doi:10.3390/jcm9072104
31. Shirakata, Y., Imafuji, T., Nakamura, T., Shinohara, Y., Iwata, M., Setoguchi, F., Noguchi, K., & Sculean, A. (2022). 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. Journal of Clinical Periodontology, 1–11. https://doi.org/10.1111/jcpe. 13694
32. Ho_mann, T.; Richter, S.; Meyle, J.; Gonzales, J.R.; Heinz, B.; Arjomand, M.; Sculean, A.; Reich, E.; Jepsen, K.; Jepsen, S.; et al. A randomized clinical multicentre trial comparing enamel matrix derivative and membrane treatment of buccal class II furcation involvement in mandibular molars. Part III: Patient factors and treatment outcome. J. Clin. Periodontol. 2006, 33, 575–583. [CrossRef] [PubMed]
33. de Queiroz Fernandes, J.; de Lima, V.N.; Bonardi, J.P.; Filho, O.M.; Queiroz, S.B.F. Bone regeneration with recombinant human bone morphogenetic protein 2: A systematic review. J. Maxillofac. Oral. Surg. 2018, 17,13–18. [CrossRef]
34. Anitua, E.; Piñas, L.; Alkhraisat, H.M. Endoret®(PRGF®) Application in the Oral and Maxillofacial Field.In Platelet Rich Plasma in Orthopaedics and Sports Medicine; Anitua, E., Cugat, R., Sánchez, M., Eds.; Springer:Cham, Switzerland, 2018; pp. 99–123.
35. De Angelis, P.; De Angelis, S.; Passarelli, P.C.; Liguori, M.G.; Manicone, P.F.; D'Addona, A. Hard and Soft Tissue Evaluation of Di_erent Socket Preservation Procedures Using Leukocyte and Platelet-Rich Fibrin:A Retrospective Clinical and Volumetric Analysis. J. Oral. Maxillofac. Surg. 2019, 77, 1807–1815. [CrossRef]
36. Eliezer, M.; Imber, J.C.; 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, 23,3423–3435. [CrossRef]
37. Pilloni, A., Schmidlin, P. R., Sahrmann, P., Sculean, A., & Rojas, M. A. (2019).Effectiveness of adjunctive hyaluronic acid application in coronally advanced flap in Miller class I single gingival recession sites: A randomizedcontrolled clinical trial. Clinical Oral Investigations, 23(3), 1133–1141. https://doi.org/10.1007/s00784-018-2537-4
38. Gangliang Huang, Hualiang Huang, Application of hyaluronic acid as carriers in drug delivery, Drug Deliv. 25 (1) (2018) 766–772, doi: 10.1080/10717544.2018.1450910.
39. Mia Krolikoski, et al., The CD44-HA axis and inflammation in atheroscle- rosis: a temporal perspective, Matrix Biol. 78-79 (2019) 201–218, doi: 10.1016/j.matbio.2018.05.007
40. W.Y.J. Chen, G. Abatangelo, Functions of hyaluronan in wound repair, Wound Repair Regenerat. 7 (2) (1999) 79–89.
41. R. Moseley, et al., Comparison of the antioxidant properties of HYAFF R ○-11p75, AQUACEL R ○and hyaluronan towards reactive oxygen species in vitro, Biomaterials 23 (10) (2002) 2255–2264, doi: 10.1016/s0142-9612(01)00360-x .

42. Dahiya, P., & Kamal, R. (2013). Hyaluronic acid: A boon in periodontal therapy. North American Journal of Medicine and Science, 5(5), 309–315.https://doi.org/10.4103/1947-2714.112473

43. Eick, S., Renatus, A., Heinicke, M., Pfister, W., Stratul, S. I., & Jentsch, H. (2013). Hyaluronic acid as an adjunct after scaling and root planing: A prospective randomized clinical trial. Journal of Periodontology, 84(7),941–949. https://doi.org/10.1902/jop.2012.120269

44. Scully, M. F., Kakkar, V. V., Goodwin, C. A., & O'Regan, M. (1995). Inhibition of fibrinolytic activity by hyaluronan and its alcohol ester derivatives.Thrombosis Research, 78(3), 255–258. https://doi.org/10.1016/00 3848(95)90876-

45. West, D. C., Hampson, I. N., Arnold, F., & Kuma, S. (1985). Angiogenesis induced by degradation products of hyaluronic acid. Science, 228(4705), 1324–1326. https://doi.org/10.1126/science.2408340

46. Pilloni, A., & Bernard, G. W. (1998). The effect of hyaluronan on mouse intramembranous osteogenesis in vitro. Cell and Tissue Research, 294(2), 323–333. https://doi.org/10.1007/s004410051182

47. David Evered, Julie Whelan, The Biology of Hyaluronan, Wiley, 1989 . [38] Erin L. Pardue, et al., Role of hyaluronan in angiogenesis and its util- ity to angiogenic tissue engineering, Organogenesis 4 (4) (2008) 203–214, doi: 10.4161/org.4.4.6926 .

48. National Research Council Committee for the Update of the Guide for the C, Use of Laboratory A. The National Academies Collection: Reports funded by National Institutes of Health. Guide for the Care and Use of Laboratory Animals. Washington (DC): National Academies Press (US) Copyright © 2011, National Academy of Sciences.; 2011.

49. Donos N, Bosshardt D, Lang N, Graziani F, Tonetti M, Karring T, Kostopoulos L. Bone formation by enamel matrix proteins and xenografts: an experimental study in the rat ramus. Clin Oral Implants Res. 2005;16(2):140-6.

50. Pilloni, A.; Rojas, M.A.; 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, 25, 5095–5107.

51. Universität Düsseldorf. G*Power.2019. Retrieved from http://www.gpower.hhu.de/

52. Nanes, B. A. Slide Set: Reproducible image analysis and batch processing with ImageJ. Biotechniques 59, 269–278. https://d oi.o rg/10. 2144/ 00011 4351 (2015).

53. Cortellini, P.; Tonetti, M.S. Clinical Concepts for Regenerative Therapy in Intrabony Defects. Periodontol. 2000 2015, 68,282–307.

54. Nevins, M.; Giannobile, W.V.; McGuire, M.K.; Kao, R.T.; Mellonig, J.T.; Hinrichs, J.E.; McAllister, B.S.; Murphy, K.S.; McClain, P.K.;Nevins, M.L.; et al. Platelet-Derived Growth Factor Stimulates Bone Fill and Rate of Attachment Level Gain: Results of a Large Multicenter Randomized Controlled Trial. J. Periodontol. 2005, 76, 2205–2215. [CrossRef] [PubMed]

55. Cochran, D.L.; Oh, T.-J.; Mills, M.P.; Clem, D.S.; McClain, P.K.; Schallhorn, R.A.; McGuire, M.K.; Scheyer, E.T.; Giannobile,W.V.;Reddy, M.S.; et al. A Randomized Clinical Trial Evaluating Rh-FGF-2/_-TCP in Periodontal Defects. J. Dent. Res. 2016, 95,523–530. [CrossRef] [PubMed]

56. Matarasso, M.; Iorio-Siciliano, V.; Blasi, A.; Ramaglia, L.; Salvi, G.E.; Sculean, A. Enamel Matrix Derivative and Bone Grafts for Periodontal Regeneration of Intrabony Defects. A Systematic Review and Meta-Analysis. Clin. Oral Investig. 2015, 19,1581–1593.

57. Kao, R.T.; Nares, S.; Reynolds, M.A. Periodontal Regeneration—Intrabony Defects: A Systematic Review from the AAP Regeneration Workshop. J. Periodontol. 2015, 86, S77–S104. [CrossRef]

58. Li, F.; Yu, F.; Xu, X.; Li, C.; Huang, D.; Zhou, X.; Ye, L.; Zheng, L. Evaluation of Recombinant Human FGF-2 and PDGF-BB in Periodontal Regeneration: A Systematic Review and Meta-Analysis. Sci. Rep.2017, 7, 65.

59. 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. Berl. Ger. 1985 2013, 44, 231–240.

60. Vanden Bogaerde, L. Treatment of Infrabony Periodontal Defects with Esterified Hyaluronic Acid: Clinical Report of 19 Consecutive Lesions. Int. J. Periodontics Restor. Dent. 2009, 29, 315–323.

61. 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;52:2–10; doi: 10.3290/j.qi.b937003)

62. Ahmed M.El-Bana, Una M. El-Shinnawi, Islam M.Attia The Effect of Hyaluronic Acid in Combination With β-Tri-Calcium Phosphate in Regeneration of Periodontal Vertical Bone Defect in Periodontitis Patients Mansoura Journal of Dentistry 2020;7(27):80-87.

63. Božić, D.; Ć atović, I.; Badovinac, A.; Musíc, L.; Par, M.; Sculean, A. Treatment of Intrabony Defects with a Combination of Hyaluronic Acid and Deproteinized Porcine Bone Mineral. Materials 2021, 14, 6795. https://doi.org/10.3390/ ma14226795