

# HISTOLOGICAL EVALUATION OF ANGELICA SINENSIS IN MANAGEMENT OF CLASS II FURCATION DEFECT (EXPERIMENTAL STUDY)

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## ABSTRACT

**INTRODUCTION:** proper management of furcation involvements has always been a demanding issue in periodontal therapy. Herbal medicine is nowadays proposed to provide novel alternative approaches for restoring defective bone. A Chinese herb known as *Angelica sinensis* (As) has various pharmacological effects as it promotes proliferation and differentiation of osteoblasts

**OBJECTIVES:** The aim of this study was to evaluate the effectiveness of *Angelica sinensis* in conjunction with  $\beta$ -tricalcium phosphate in the management of class II furcation defects in dogs

**MATERIALS AND METHODS:** This randomized study was carried on 6 clinically healthy mongrel dogs. 24 grade II critical-sized furcation defects were surgically created in the mandibular third and fourth premolars. 12 defects were filled with Beta-tricalcium phosphate ( $\beta$ -TCP) bone graft only (control group). While in the other 12 defects  $\beta$ -TCP was mixed with *Angelica sinensis* powder (experimental group). The defects were covered by collagen membrane, The dogs were sacrificed at 4 and 8 weeks postoperative.

**RESULTS:** The histological analysis has revealed better regenerative features regarding alveolar bone, periodontal ligament and cementum in the experimental groups when compared to control groups.

**CONCLUSIONS:** *Angelica sinensis* herb could enhance the periodontal regenerative potential of  $\beta$ -TCP bone graft.

**KEYWORDS:** *Angelica sinensis*, bone graft, furcation,  $\beta$ -TCP

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## INTRODUCTION

Periodontal diseases are defined as multifactorial, polymicrobial infections involving the destruction of tooth-supporting tissues including alveolar bone, periodontal ligament and cementum(1).

Progression of periodontitis leads to furcation involvement which is the pathologic resorption of the supporting alveolar bone. Despite the fact that teeth with furcation involvement can be kept long under the proper maintenance care, the treatment of furcation involvement has always been a clinical challenge since the anatomy of the furcation area always complicates the ability for proper hygiene and surgical operation (2).

Various modalities for class II furcation defects such as scaling and root planing and systemic or local antimicrobial have been used together with surgical periodontal therapy(2, 3) . Laser therapy and photodynamic therapy have also been utilized(3).

Surgical procedures, however, included surgical open flap debridement, regenerative therapy, root resection and hemisection(2, 4) .

Guided tissue regeneration (GTR) allows selective progenitor cells to fill the defect site and prevent the in-growth of gingival epithelium and the connective tissue cells (5, 6). Recent developments showed that alloplast bone graft and bioresorbable GTR barrier membranes have adequate biologic response and handling properties(7)

$\beta$ -tricalcium phosphate ( $\beta$ -TCP) is an alloplast with a porous structure. This structure provides the desired biologic properties, i.e., osteoconductivity and total resorbability. It releases calcium and phosphate ions resulting in new bone formation(8).

Collagen membranes are formed of fibers which give them structural elasticity throughout the crystalline stage of bone regeneration. These

properties guarantee good tissue integration and adequate wound healing. The collagen micro-architecture and cross-links outline its structural durability, stiffness and degradation time(9).

The American Academy of periodontology workshop on furcation treatment has highlighted that the Future research efforts should be mainly approached to clinical trials that test novel regenerative approaches that emphasizes on histologic demonstration of periodontal regeneration(2) .

The root of *Angelica sinensis* (Oliv.) Diels has been utilized for many years in traditional Chinese, Korean, and Japanese medicine(10). The whole root is brown in color and cylindrical in shape with many branch from its lower end (11).

The active constituents of *A. sinensis* roots which are responsible for their bioactivities include polysaccharides, organic acids and phthalides. Ferulic acid and Z-ligustilide (11-13) . In vitro and vivo studies of *A. sinensis* showed variety of pharmacological activities, including protection of the heart, enhancement of immune function, anti-arrhythmic, anti-atherosclerotic, and prevention of myocardial infarction events by inhibition of smooth muscle inflammation and platelet aggregation.(11, 14, 15) .It also shows anti-inflammatory, anti-cancer, immunomodulatory, neuroprotective, anti-oxidative, anti-hepatotoxic and renoprotective activities (16)

Several studies have shown that *Angelica sinensis* has both osteogenic and angiogenic effects resulting in an increase bone formation and promotion of fracture healing (10, 17). *Angelica* polysaccharide has been reported to induce proliferation and osteoblast differentiation of mesenchymal stem cells(18, 19). Based on these recent researches, the present study attempted to further evaluate the potential of *Angelica Sinensis* herb to promote periodontal regeneration in surgically induced critical-size Class II furcation defects in dogs.

The null hypothesis proposed that there is no significant difference between the furcation defects managed with or without *Angelica sinensis*.

## MATERIAL AND METHOD

### Material

#### Study animals

The experimental protocol was given the approval from The Animal Research Committee of Alexandria University (IRBNO:00010556-IORG0008839).

Inclusion criteria: Systemically healthy dogs, 15-20 months old and weighing between 14- 20 Kg.

Exclusion criteria: dogs with any systemic diseases or detectable injuries.

#### Study design

A randomized study was carried on 6 dogs with the defects created at the mandibular third and fourth premolars ( $P_3, P_4$ )

Gpower version 3.1.9.2 was used to estimate the sample size. Considering significance level of 95% ( $\alpha=0.05$ ), the minimum required sample size is found to be 6 defects per group (number of groups 4) (Total sample size =24 defects).

#### Grafting materials

1- Bone grafting material (Medbone Biomaterials Sintra, Portugal).

Adbone<sup>®</sup> TCP (500-1000  $\mu$ m 0.5g)  $\beta$ -tricalcium phosphate ( $\beta$ -TCP) alloplast

2- Collagen membrane

Hypro-Sorb<sup>®</sup> Collagen Membrane (30x40x0.2mm) Type I Atelo -collagen from the bovine Achilles tendon (Bioimplom GmbH, Giessen, Germany)

*Angelica sinensis* (Active Herb company, Suite E, San Diego)

Dong Quai, Chinese *Angelica Sinensis* Extract which is certified by Chinese GMP and enforced by FDA with ferulic acid as the active ingredient.

### Methods

#### Surgical procedures

The animals were given sodium thiopental intravenous injection (Sandoz GmbH Biochemistries, Ostrich, Austria) (13 mg/kg) for general anesthesia. 1:100,000 epinephrine and 2% lidocaine HCL (Novocol Pharmaceutical of Canada, Inc. Cambridge, Ontario, Canada) were locally infiltrated at the surgical sites using metal dental syringe. Opposite to the lower third ( $P_3$ ) and fourth ( $P_4$ ) premolars sulcular incision was carried out to expose the furcation area. Buccal mucoperiosteal flaps were then fully reflected. Grade II furcation defects of 4 x 5 mm dimensions measured by Williams Periodontal Probe were created under copious irrigation using carbide bur in  $P_3$  and  $P_4$  of each dog(20-22). At the apical border of each defect two reference notches were drilled on the mesial and distal root surfaces at the base of each defect(23).this is essential for the future histological examination. Universal curette was used for root planning followed by Ethylenediaminetetraacetic Acid (META BIOMED CO, LTD, Korea) for 15 seconds for root conditioning. 12 of the created defects were filled with  $\beta$ -TCP alone and the other 12 created defects were filled with equal scopes of As granules mixed with  $\beta$ -TCP in the ratio of 1:1. Collagen membranes were trimmed to extend 2-3 mm at each side of the defect. They were then placed over the filled defects. Since the collagen membranes were properly adapted on bone and root surfaces, there was no need for suturing the membranes. Coronal repositioning of the flap over the grafted sites. Simple interrupted suturing using 3-zero

silk sutures (Johnson & Johnson, NJ) ensured complete defect closure

#### Postoperative care

In the first day, Antibiotic of intramuscular injection of ampicillin (1 g) and Ibuprofen non steroid anti-inflammatory 600 mg (Brufen 600 mg Abbot, Germany). For the next 7 days, the dogs were given the same antibiotic and the anti-inflammatory drugs but mixed with their food. Suture removal was carried out after 12 days. During the two observational periods, the animals were kept in collective kennels, one per kennel, proper ventilation and light/dark cycle (12/12h) was maintained. The animals were routinely checked for weight loss, gingival and soft tissue inflammation.

#### Animal euthanasia

Intravenous thiopental sodium was used for euthanasia. After 4 weeks, three dogs were euthanized and after 8 weeks, the other three dogs were euthanized.

#### Histological procedure

Euthanasia was followed by dissection of the mandibles which were then fixed in 10% neutral buffered formalin. Running water was then used for complete rinsing of the dissected specimens. Several baths of 5% trichloroacetic acid were used to decalcify the specimens. During a period of 3 weeks the acid was changed every 3 days. After complete decalcification, all acid traces were washed off by running water for a period of 12 hours. Ascending concentration of ethyl alcohol (50%,70%,90%) has gradually dehydrated the specimens. The specimens were passed from alcohol through two changes of xylene. The specimens were then infiltrated with paraffin into a constant temperature furnace regulated to about 60 degrees Celsius It is essential that this step lasts for 12-24 hours for complete infiltration of the specimens by paraffin. The specimens were then embedded in paraffin waxed blocks for mesio-distal sectioning. Serial sections of 5 microns thickness mesiodistal were sliced using rotatory microtome. The sections were placed on clean glass slides coated with albumin adhesive layer. The slides were placed in 60-degree Celsius constant temperature furnace to ensure adherence of sections to the slides. Hematoxylin and Eosin (H&E) and Gomori trichrome were used to stain the sections which were then investigated under light microscope(24, 25).

Concealment of group allocation from the researcher performing the histological examination ensured a single-blind study and made the results of the study less likely to be biased.

#### Histomorphometric quantitative analysis

Image J 1.46 r software (26) was used to measure the height of newly formed bone in the furcation region on the histological specimens.

To get quantitative measures from each block, four slides of tissue from different depths were selected. Using the same magnification power, one photograph from each slide showing the furcation region, parts of PDL and parts of the adjacent two roots were taken. The best photograph was then used for quantification in the software.

On each photograph, a line connecting the created notches on the mesial and distal roots were drawn. A second line was then made from the most coronal part of the furcation till the first drawn line. From set scale at analyze, the readings were changed from pixel to millimeters at scale of 5 mm. A third straight line connecting the most coronal end of the newly formed bone to the first line of the notches was then drawn. This line represented the length of formed bone.

#### Statistical analysis

Measurements quantified from the histomorphometric analysis were analyzed by mean and standard deviation. The level of Significance of the results was adopted at the 5% level

## RESULTS

### 1.Histological results

#### First observation period (4 weeks)

##### Experimental group

Small rod-shaped bone trabeculae extended from the side of the regenerating bone at the periphery of the defect. Union between the different segments of the forming bone was traced. An important finding in this group was the profound blood supply and proliferating blood vessel in the different regions of the defect (Figures 2A&B). Here a striking relation was noted between the proliferating blood vessels,  $\beta$ -TCP, As and the formed bone.  $\beta$ -TCP particles formed a network enclosing the other three components and outlined by thin ribbons of newly forming bone (Figure 2C). Another observation was the extension of bone formation to occupy the notch regions made on the root surfaces, (Figures 2D&E). Also, bone formation was traced extending from the native bone at the base of the defect towards its coronal boundaries, (Figure 2F).

##### Control group

Limited amount of forming bone was traced at the defect base and constituted irregular trabeculae originating from the defect base and traversing coronally (Figure 3A).

Adjacent to the regenerating cementum irregularly organized cementoblasts were seen adjacent to the forming cementum and close to the randomly distributed fibroblasts of the forming PDL. Also, small osteoblast cells appeared arranged on the periphery of the forming trabeculae, (Figures 3 B&C)

In sections of this group, no evidence of bone formation was noted at the notch depressions on the roots bordering the lateral sides of the defects  
 Second observation period (8 weeks)

Experimental group

An evidence of osteon formation was noted in the mass of the forming bone reflecting start of compact bone transformation, (Figures 4A&B). Remodeling lines were also seen near the base of the defect where they separated the different segments forming the trabeculae, (Figure 4C).

In this group, the density of the bone formed in the root notches appeared greater than that formed in the former groups, (Figure 4D). The fibers of the regenerating PDL appeared highly organized and arranged in dense groups and traversed by many blood vessels from the adjacent regenerating alveolar bone through Volkmann's canals, (Figure 4E).

Control group

At the defect base, bone trabeculae of moderate thickness could be traced projecting and traversing towards the defect center and enclosing few figures of  $\beta$ -TCP particles, (Figure 5A).

At higher magnification osteoblasts cells were seen mapping the forming bone trabeculae. They were slightly apart from each other and their size was not comparable to that of osteoblasts of the 8 weeks experimental group, (Figure 5B).

Towards cementum, Remodeling features were noticed among the PDL fibers. Cementum reformation was also noted, (Figure 5C).

2.Histomorphometric analysis

4 weeks postoperatively, the height of interradicular bone in the furcation defects managed with  $\beta$ -TCP and As were  $3.0 \pm 0.09$ mm. The defects filled with  $\beta$ -TCP only showed lower results of  $2.5 \pm 0.15$ mm with statistical difference between them ( $p < 0.001$ ). 8 weeks postoperatively the bone height of the test group was  $3.6 \pm 0.11$ mm which was also greater than the control group of  $3.0 \pm 0.25$ mm with statistically significant difference of 0.0001 (Table 1)

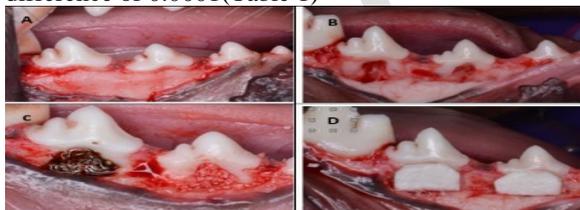


Figure1: (A) After sulcular incision, reflection of full thickness mucoperiosteal flap (B) the appearance of the surgically created critical sized defects of P3 and P4. (C) Placement of  $\beta$ -tricalcium phosphate in the created defect of lower right P3 (control group) and As and  $\beta$ -tricalcium phosphate in the created defect of lower right P4 (experimental group) (D) Application of collagen membrane over the grafted defects

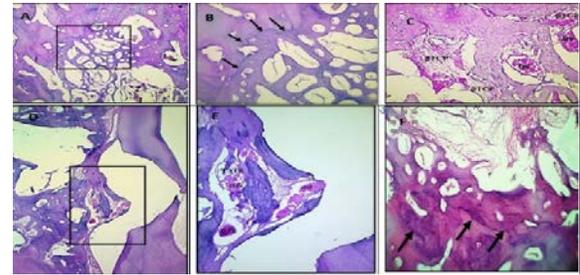


Figure 2: A&B) showing formation of thin trabecular bone in the central regions of this specimen projecting towards the center of the defect. B shows higher magnification of the boxed area in A revealing the formed thin trabeculae and their union with the bone formed at the defect periphery (black arrows). H&E stain, A:X40, B:X 100. C) showing profound blood supply among the forming trabeculae (bv) an intimate relation between  $\beta$ TCP, *Angelica* patches (An), the proliferating blood vessels and the regenerating bone. H&E stain 100 D&E) showing extension of new bone formation in the root notches at the lower boundary of the defect. B is higher magnification of the boxed area in A. Note the network of  $\beta$ TCP, *Angelica* (An) and proliferating blood vessels (bv). H&E stain, D:X40, E:X 100 F) showing the line of union between the regenerating bone and that of the defect base (black arrows). H&E stain, X 100

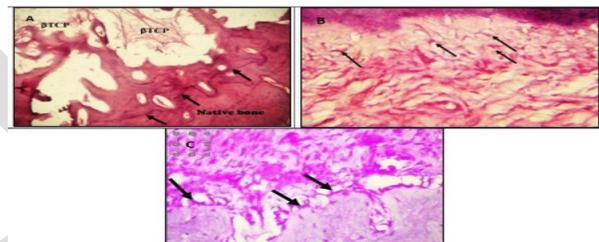


Figure 3 A) showing the limited amount of thin irregular trabeculae emerging from the native bone at the defect base and enclosing particles of  $\beta$ TCP. The arrows show the union line between both. H&E stain, X100. B) shows irregularly organized cementoblasts towards the newly forming cementum (thin black arrows) and facing disorganized PDL fibers and fibroblasts. C) shows small osteoblast cells adjacent to the regenerating trabeculae (thick black arrows), B&C:H&E stain, X400

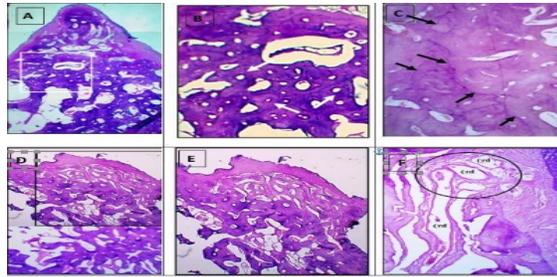


Figure 4 A&B) shows formation of dense regenerating bone in most of the defect regions extending from the bifurcation till the base B: shows higher magnification of the boxed area in A revealing osteon formation in the dense regenerating bone (black arrows) H&E stain, A:X40, B:X 100. C) showing remodeling of the bone formed at the defect base and the line of their union (black arrows). H&E stain, X: 100 D&E) showing greater density of forming bone at the notch region on the root limiting the end of the defect. E: is higher magnification of the boxed area in D revealing the perfect organization of trabecular bone in this area H&E stain, D:X:40, E:X 100 F) showing highly organized PDL fibers arranged in dense groups and traversed by many large blood vessels (bv) from the adjacent regenerating alveolar bone through Volkmann's canals (circle) H&E stain, X 100

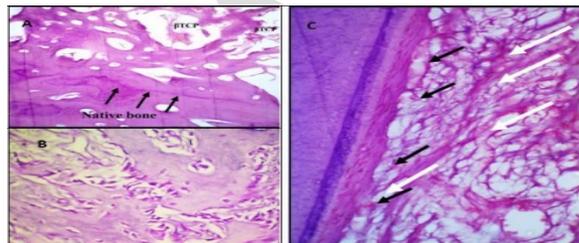


Figure 5 A) showing the union between the regenerating bone trabeculae at the defect base with the native bone (black arrows). Few particles of  $\beta$ TCP are seen among some of the trabeculae. H&E stain, X 100 B) moderate sized osteoblast cells bordering the regenerating trabeculae. They are slightly separated from each other and do not form a continuous line on these trabeculae, H&E stain, X 400 C) showing slightly flattened cementoblasts (black arrows) differentiating adjacent to the border of the forming cementum The fibers of the forming PDL exhibit remodeling features with thickness variation and specific directivity towards cementum surface (white arrows). Trichrome stain, X: 400

**Table (1):** Comparison between the two studied groups according to the height of newly formed bone in furcation region

| Height of the newly formed furcation region in mm | (mean $\pm$ SD) | P value       |
|---|-----------------|---------------|
| <b>4 weeks</b>                                    |                 |               |
| Control group                                     | 2.5 $\pm$ 0.15  | <b>0.001</b>  |
| Test group  | 3.0 $\pm$ 0.09  |               |
| <b>8 weeks</b>                                    |                 |               |
| Control group                                     | 3.0 $\pm$ 0.25  | <b>0.0001</b> |
| Test group  | 3.6 $\pm$ 0.11  |               |

**DISCUSSION**

Periodontitis causes tissue destruction that gradually extends to furcation space resulting in bone loss. In periodontal therapy, furcation management is always considered a demanding task as the peculiar anatomy of the junction often makes it difficult to completely remove the local factors in the interradicular area(27, 28).

The limitation of the currently used bone grafts has necessitate the need to find substitute products. Donor site morbidity of autogenous grafts and the risk of disease transfer of allografts and xenografts are among the restrictions associated with different grafting materials(29, 30). Also, the exhaustive processing procedures such as lyophilization, freeze drying and sintering were found to adversely affect the structural integrity and osteogenic properties of several bone grafts(31, 32). These many drawbacks have resulted in ongoing search for alternative products in bone grafting procedures

Herbal medicine provides a cost-effective as well as a natural mode of management to many chronic diseases and has proven to have the potential of tissue regeneration. *Angelica sinensis* is regarded as one of the herbs that shows osteogenic regenerative effect. This experiment investigates the regenerative potential of *As* in conjunction with an osteoconductive bone grafting material. The histological analysis at both observational periods has revealed higher bone formation in the experimental groups with greater bone density in the root notches compared to the control groups. This high osteogenic potential was explained by several mechanisms in many studies.

Wang D et al.(33)explained how *Angelica sinensis* ligustilide component may be used for preventing and treating abnormal bone resorption. It suppresses the formation and activity of osteoclast cells via the inhibition of receptor activator of nuclear factor- $\kappa$ B (RANK) expression and downregulation of the messenger RNA (mRNA) expression of osteoclast-specific genes. This was supported by another research that also showed that ligustilide stimulated osteoblast differentiation(34).

Other studies has showed that As can enhance bone formation and promote fracture healing(35).This is due to its both osteogenic and angiogenic properties, through stimulation of substances such as vascular endothelial growth factor (17).In addition , *Angelica sinensis* polysaccharide has reported to activate specific signaling pathway resulting in differentiation of rat bone marrow mesenchymal stem cells and promotion of bone regeneration (18).

The placement of As in a herbal formula compositions on ligature-induced periodontitis has shown analeptic effects on the periodontal destruction through inhibiting alveolar bone resorption .It decreased the osteoclastogenesis and promoted bone and periodontal regeneration(36)..

The height of newly formed bone in *Angelica Sinensis* group in the furcation region were  $3.0\pm 0.09$  and  $3.6\pm 0.11$ mm 4weeks and 8 weeks respectively which was in accordance to Zohery et al. (23) who investigated the use Egyptian propolis. After the 4 weeks observation period, the bone height for Egyptian propolis and nanohydroxyapatite groups were 3.89 to 4.68 and 3.91 to 4.42 respectively. Comparable results were also reported in another study which involved the effect of plasma-rich plasma and bioactive glass in management of class II furcation defects(37). The height of interradicular bone after 45 days for the control group involving bioactive glass only and for the test group where plasma-rich plasma was added were 3.68 and 4.60 respectively.

From all the above information, *A. sinensis* can be safely utilized in conjunction with bone graft materials. However, further studies are still recommended to evaluate its use in other periodontal defects and to understand the mechanism behind its regenerative potential.

## CONCLUSION

*Angelica sinensis* is a biocompatible herb exhibiting no adverse or allergic effect. Confirmed by the histological evaluation, *Angelica sinensis* possesses high periodontal tissue regeneration potential as it stimulates bone, cementum and PDL regeneration. Out of the promising results *Angelica sinensis* has shown, it is expected to be well established in the field of guided tissue regeneration.

## CONFLICT OF INTEREST

No conflict of interest.

## FUNDING:

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