The Use of Oleozon Gel in the Treatment of Surgically Induced Two-Wall Osseous Defects in Mongrel Dogs (Histological Study)

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Abstract: Background and Objectives: This experimental study was conducted to investigate the resulting histological regeneration after the use of Oleozon gel in the treatment of induced periodontal defects in Dogs. Sixteen male mongrel dogs were included in this study. Their ages ranged from 18-24 months and their weights from 12-15 kg. All animals were systemically healthy. Thirty two surgically induced deep two-wall osseous defects (more than 5 mm) of matched severity were induced bilaterally around the lower premolar teeth of each dog, using a small size round or fissure carbide surgical bur. The defects were divided into; Group I which included sixteen surgically induced defects on one quadrant of the sixteen animals where they received Oleozon gel, in addition to Group II which included sixteen surgically induced defects on the opposite quadrants where they did not receive any regenerative treatment and had been left to self heal . Group I demonstrated a significantly higher bone formation and regeneration of the attachment apparatus than was seen in group II. It was concluded that the use of Oleozon gel led to significantly greater gain of clinical attachment and hard tissue formation.
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1. Introduction

Currently, ozone is being discussed in dentistry as a possible alternative oral antiseptic agent. Its high antimicrobial power against most pathogens, without resistance development, has been reported not only for gaseous ozone, [1,2] but also for ozone in aqueous solution. [3-6] In concentrations currently used in dentistry, ozone gas has been found to decrease the viability of oral cells significantly. [7] In comparison, aqueous ozone revealed a high level of biocompatibility to fibroblasts, cementoblasts, and epithelial cells [8,9], which suggests its use against oral infectious diseases, where it comes into contact with resident oral cells, e.g. periodontium and gingival tissue.

Ozone is a gas that has a very high reactivity, and it is very unstable, with a half life of just a few minutes. It cannot be stored, so has to be made on demand when required. In contrast, when it is dissolved in an oil base, it has a life span that could be measured in years. It chemically reacts with oil, and forms long complex molecules. [10, 11]

Oleozon is pure olive oil that has undergone ozonization using a steady flow of ozone-oxygen mixture in the ratio of 5:95 % until olive oil transforms from the greenish-colored liquid status to the whitish gel status. [11]

Due to the germicidal action of Oleozon, as well as its oxygenating power, that favors the tissue regeneration, it was applied in the treatment of alveolitis following surgical extraction of lower third molar. More rapid healing was achieved in patients treated with Oleozon in comparison with those treated with Alvogil. [12, 13]

Periodontal disease is a local inflammation initiated by dental plaque bacteria. In chronic periodontitis, the presence of periodontal pathogens such as Porphyromonas gingivalis and Tannerella forsythensis are necessary for initiation of inflammation; however, the progression of periodontal disease depends on the host response to various bacterial products and components. The destruction observed in periodontal disease is the result of an improperly regulated innate immune response to bacterial infection characterized by the recruitment of inflammatory cells, generation of proinflammatory proteins such as cytokines and chemokines, and activation of osteoclasts.[14-17]

Therefore, the aim of the present study was to evaluate histologically the outcomes of the Oleozon in the treatment of induced periodontal defect in Mongrel Dogs.

2. Materials and Methods

Sixteen male mongrel dogs were included in this study. Their ages ranged from 18-24 months and their weights ranged from 12-15 kg. All animals were systemically healthy. A total of sixteen surgically induced deep two-wall osseous defects (more than 5 mm), of matched severity, were induced bilaterally around the lower premolar teeth of each dog using a small size round or fissure carbide surgical bur. In each animal one defect was treated with Oleozon gel (Group I), while the opposite defect did not receive any regenerative treatment (Group II/Control). The control defects were treated with sterile saline solution and had been left to heal spontaneously. Animal care and experimental procedure were performed in accordance with guidelines of the review board of Qassim University animal and experimental lab.

Surgical Procedures:

The animals were anaesthetized with intravenous thiopental sodium (25 mg/kg induction, 150 to 200 mg maintenance doses given intermittently to maintain deep anesthesia). Sulcular incision on the buccal and lingual side of the tooth was followed by elevation of the mucoperiosteal flaps buccally and lingually in the mandibular first premolar area. Two-wall osseous defects were created between the lower premolars of matched severity (more than 5 mm). The root surfaces were instrumented to remove all residual cementum at the site of defect creation, thus leaving the dentine surface completely denuded. A notch was created on the bare dentin surface of the premolars just coronal to the surgical defect. On one quadrant the defects received Oleozon gel [*] (Group I), while for the opposite quadrant the defects did not receive any regenerative treatment, only sterile saline solution (Group II).

The flaps were adapted to their original positions and interrupted sutures were performed using 3-0 black silk[†], a pain killer was also given (Voltaren [‡] 25 mg IM) on the first day postoperatively. The animals were housed and fed with soft diet for a week (to minimize the possibility of trauma to the site of the operation) then on standard pellet diet and water ad libitum. They were kept under controlled environmental conditions of temperature: 24±2°C, humidity: 50-55%, and natural 12-h light/dark cycle.

At 1, 3, 6 and 9 month intervals post operatively; eight animals were sacrificed using an overdose of thiopental sodium IV injection.

Histological procedures

Tissue blocks including bone, experimental teeth, and surrounding soft tissues were obtained after euthenization. The specimens were fixed in 10% buffered natural formalin for two weeks, and then decalcified in 5% Trichloroacetic acid. Serial sections of 7 μm were cut in the mesiodistal plane parallel to the long axis of the roots representing most of the induced defect area. Nearly half the number of the sections was stained using Hematoxylin and Eosin (H&E) stain, while the other half were stained using Trichrome stain. [(18)]

3. Histological Results

Group I: Oleozone group

One month post operatively the residual bone was found to lie apical to the notch and is covered by a thick layer of granulation tissue. Fig (1) **Three month** post operatively reveals bone regeneration radiating from old bone in a coronal direction reaching the notch area. The newly regenerated bone is attached to newly regenerated cementum in the notch area by newly regenerated periodontal ligament fibers. A thicker granulation tissue covers the regenerated bone. A newly regenerated dental papilla is seen coronally in the interdental area. The surface of the papilla is found to be epithelized Fig (2). The granulation tissue in the notch area is highly cellular and vascular. Starting osteoid is seen in the granulation tissue proximal to the newly regenerated bone Osteoblasts cover the newly regenerated bone while cellular cementum covers the root surfaces in the notch area. Fig (3) **Six months** post operatively shows the newly regenerated bone completely filling the defect area coronally. Newly regenerated attachment apparatus is also seen inside the notch. Newly regenerated periodontal ligament fibers (PDL) attach the newly formed bundle bone to newly regenerated cementum. The bone is covered coronally by a newly regenerated interdental papilla which is covered by a thin epithelial layer. Fig (4) Higher magnification of the newly regenerated dental papilla shows the regeneration of interseptal fibers attaching neighboring teeth. The fibers become embedded in the cementum of one tooth, running above the level of the newly regenerated bone to become attached to the cementum of the neighboring tooth. Functionally oriented PDL groups of fibers can also be seen as oblique group and dentoalveolar group that connects root cementum and interdental bone.The regenerated dental papilla is covered by surface epithelium Fig (5). **Nine months** postoperatively shows nearly complete bone regeneration reaching the cervical area. Newly regenerated oriented periodontal ligament fibers (PDL) are attached to regenerated bone and the newly regenerated cementum and to typical newly regenerated interdental papillae. Fig (6)

* Nour Advanced Technologies, 6th October, Egypt.
† Ethicon, Johnson & Johnson, São Paulo, Brazil.
‡ Novartis Pharma, Switzerland

Group II: Control group

One month post operatively reveals the residual bone apical to the notch and is covered by a thick layer of granulation tissue Fig (7&8). **Three month** post operatively shows residual bone apical to the notch area covered by granulation tissue which shows surface epithelization. Fig (9&10) **Six month** post operatively reveals bone regeneration reaching the notch area. A flat newly regenerated interdental papilla containing disoriented periodontal ligament fibers covers the regenerated bone .The surface of the regenerated papilla is epithelized. Nine months postoperatively reveals bone regeneration reaching a level near to the notch area. A regenerated flat dental papilla that shows surface epithelization is recognized between roots of adjacent premolars. The bulk of the papilla is made of interlacing PDL fibers. Newly formed cementum covered some areas of the root where new attachments of the regenerated PDL was inserted Fig (11). **Nine months** postoperatively reveals bone regeneration reaching nearly the notch level. A newly regenerated interdental papilla containing disoriented interlacing PDL fibers covers the regenerated bone. The surface of the regenerated papilla is epithelized. Fig (12)

Group I

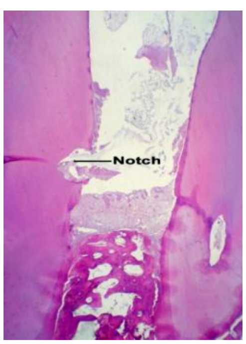

Fig. (1): Photomicrograph after 1 month showing healing after oleozone treatment. The residual bone is covered by a thick layer of granulation tissue below the notch. H&E stain (X40)

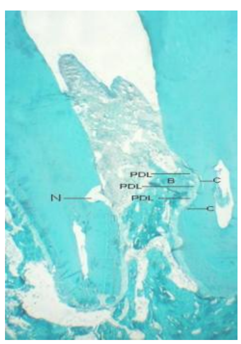

Fig. (2): Photomicrograph after 3 months showing healing after ozone treatment. It shows bone regeneration reaching the notch area (N). The newly regenerated bone (B) is attached to newly regenerated cementum (C) in the notch area by newly regenerated periodontal ligament fibers (PDL). A thicker granulation tissue covers the regenerated bone. A newly regenerated dental papilla is seen in the interdental area coronally. Surface epithelium (SE) Trichrome stain (X40)

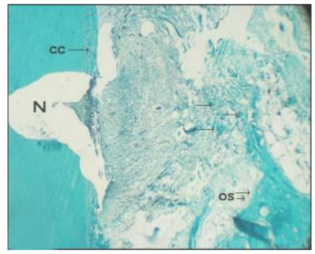

Fig. (3): Higher magnification of the previous Photomicrograph showing the cellular vascular granulation tissue in the notch area. Starting osteoid is seen in the granulation tissue proximal to the newly regenerated bone (arrow heads) Osteoblasts (OS) cover the newly regenerated bone. Cellular cementum (CC) covers the root surfaces in the notch area. Trichrome stain (X100)

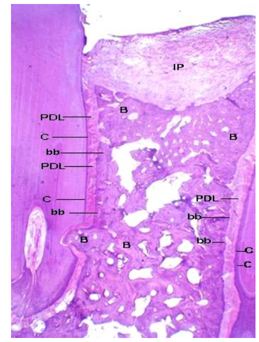

Fig. (4): Photomicrograph after 6 months showing newly regenerated bone filling the defect area coronally in addition to the inside of the notch. Newly regenerated periodontal ligament fibers (PDL) attach the newly formed bundle bone (bb) to newly regenerated cementum (C). The bone is covered coronally by a newly regenerated flat interdental papilla (IP). The interdental papilla (IP) is covered by a thin epithelial layer. H&E stain (X40)

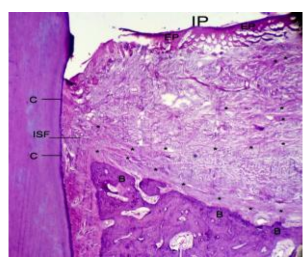

Fig. (5): Higher magnification of the previous Photomicrograph for the area of the newly regenerated interdental papilla (IP) showing the newly regenerated interseptal fibers (*) attached to cementum (C) of one tooth passing over the newly regenerated bone (B). Epithelium (EP) covers the newly regenerated interdental papilla (IP). H & E stain (X100)

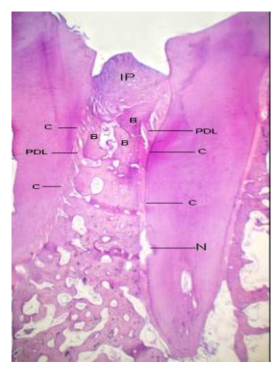

Fig. (6): Photomicrograph after 9 months showing nearly complete bone regeneration reaching the cervical area. Newly regenerated oriented periodontal ligament fibers (PDL) are attached to bone (B) and the newly regenerated cementum (C). Newly regenerated interdental papillae (IP). H & E stain (X40)

Group II (Control group)

Fig. (7): Photomicrograph after 1 month postoperatively showing healing of the residual interdental bone reaching nearly the apical thirds of the adjacent teeth. H&E Stain (X40)

Fig. (8): Photomicrograph after 1 month postoperatively, showing a thick layer of granulation tissue covering the residual interdental bone. Trichrome stain (X40)

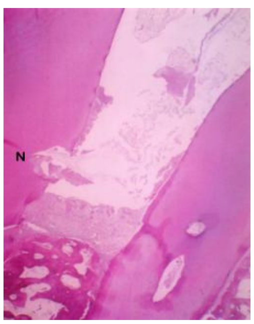

Fig. (9): 3 months postoperatively, showing residual interdental bone at apical thirds of adjacent teeth and apical to root notch A thick granulation tissue covers the residual bone. H&E (X40)

Fig. (10): Photomicrograph after 3 months, showing absence of bone formation. The residual interdental bone is covered by a fibrous granulation tissue infiltrated by inflammatory cells. Epithelization of surface area (EP). Trichrome stain (X40)

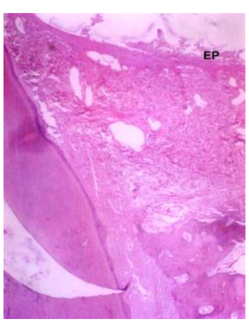

Fig. (11): Photomicrograph after 6 months, showing bone regeneration reaching the level of the notch area. A flat newly regenerated interdental papilla containing interlacing fibers covers the bone. Note surface epithelization (EP). Regenerated cementum is seen covering patrs of the root surface (arrows) where regenerated PDL fibers are inserted. H&E stain (X100)

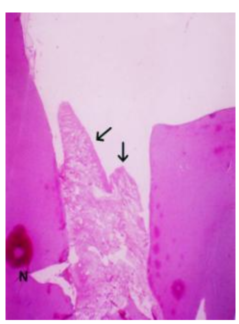

Fig. (12): Phmicrograph after 9 months postoperatively, showing residual bone nearly at the notch level. Newly formed interdental papilla between roots of adjacent teeth coronally above the level of the notch revealing surface epithelization (arrows). The bulk of the papilla is made of interlacing fibers. Areas of regenerated cementum is seen covering parts of the root ares where regenerated PDL fibers are inserted(arrow heads) H & E stain (X40)

4. Discussion

The present study investigated the effect of the use of Oleozon gel in treatment of induced periodontal defect in mongrel dogs.

Periodontal regeneration refers to the formation of alveolar bone, cementum and a new functional periodontal ligament. [19] This requires an orchestrated sequence of biological events for its successful outcome. Therapeutic modalities that attempt to enhance these biological events such as cell migration, adherence, growth and differentiation have the potential to increase the success and predictability of periodontal regenerative procedures. [20]

Periodontal diseases are prevalent diseases that involve chronic and progressive destruction of the tooth supporting tissues, mainly the alveolar bone and periodontal ligament (PDL). These diseases often lead to tooth loss. It is generally accepted that periodontal disease does not result from direct tissue destruction by pathogenic bacteria. Rather, the destructive potential of the host immune response leads mainly to signs and symptoms of these diseases. [21,22]

The approach chosen for the current study (i.e. acute model), has been chosen for several reasons; the standardized surgical defect size in the acute model allows equal conditions for healing. In addition, the surgically created defects are less time and money consuming. In the present study, surgical removal of the buccal plate of bone and the whole interdental bone, reaching nearly till the apical third for the creation of such a defect, created a very deep empty crater. The craters were subsequently filled by Oleozon for the study groups or left empty in the control group. The removal of such a large bony mass with the surrounding soft periodontal tissues is considered a major challenge for the regenerative ability of any biological mediator even when used in combination therapy. Several authors have claimed that during defect preparation and conventional root debridement, most if not all of cementum is eliminated, thus resulting in a root surface condition very similar to those seen in acute defects. [23-25] In the present study standardization the defect size and configuration was done, in addition the operator always made sure that all residual cementum in the defect area was completely eliminated.

The chosen time intervals at 1, 3, 6 months post operatively were selected to cover the different phases expected in the healing defect. Furthermore, such intervals were in accordance with other investigators who suggested the ideal time points for canine biopsies. [26] At these time intervals, bone remodeling which occurred subsequent to the administration of the periodontal regenerative therapy (Oleozon), involved several events. During these time intervals, osteoclasts worked locally for only a short time post operatively to resorb sequentially old bone, followed later on by osteoblasts that laid down new bone. The duration of this cycle is referred to as sigma which has been estimated to be four months in dogs. [27] The 9 months intervals were added to evaluate the complete regeneration of interdental papillae and periodontal ligament groups of fibers.

Immune modulation affecting treatment strategies for these diseases may be also considered. In this respect Ozone can modulate the immune system as it increases and activates body's own antioxidants and radical scavengers, due to its immunomodulating effects. It is therefore utilized in the treatment of various Allergic diseases. [21, 28]

Ozone is also a powerful antioxidant and radical scavenger [29], where direct contact of Oleozon gel with host cells leads to decrease release of reactive oxygen species as a part of the immune response. Excessive production of reactive oxygen species is one of the pathological features in the periodontal lesion and leads to damage of the periodontal tissue by oxidizing DNA, lipids, and proteins. [23,29-31]

In this current study the histological results of the Oleozon group revealed the regeneration of new bone coronal to the primarily induced defect attached to the tooth root by a viable attachment apparatus as early as three months. By six months, the Oleozon group showed the regeneration of the complete attachment apparatus including newly regenerated bone, newly regenerated oriented PDL fibers, and newly regenerated cementum in addition to a newly regenerated flat interdental papilla reaching the cervical and coronal areas respectively. This tremendous regenerating potential of Oleozon may be due to the ability of ozone to induce the production of cytokines known for their superior healing and regenerative abilities. This theory is in accordance with Bocci V. 1994 [32] who reported that ozone activates the immune system to produce more white blood cells, and many cytokines such as bone morphogenic protein, interferon, interleukin-2, and tumor necrosis factor, hence improving the healing process. These cytokines such as morphogenic protein are of critical values responsible for the unique ability to induce cartilage and bone formation and enhance their rate of formation. [10]

Also ozone was found to increase the distendability of the membrane of the red blood corpuscles, leading to an increase the blood flow even through the ischemic tissues, hence provides tissues with nutrients, oxygen, inflammatory cytokines, and white blood cells needed for healing, and as well as, increases washing action of the waste products out of the tissues and it increases the oxygen carrying capability of the red blood corpuscles. [10] In accordance with the present study, Holmes 2002 [33] found that the use of ozonized oil into a periodontal deep periodontal pocket in conjunction with thorough scaling and repeated every day achieved complete resolution of an infected pocket in about 2 days in a pockets of 3-4mm depth while gave a similar effect in about 7 days in deeper pockets, and he explained this results that ozonized oil have the ability to stimulate the reparative cellular mechanisms.

The final results seen in this study at the nine months period revealed the progress of the attachment apparatus regeneration showing actual regeneration of bone till interdental crest and the reattachment of all principal and gingival fibers of periodontal ligament. The newly regenerated bone was always surrounded by more newly regenerated granulation tissue, thus promoting further regeneration in distant areas coronal to the defect in an attempt to regenerate the lost interdental papilla together with the complete regeneration of the attachment apparatus. These superior regenerative capabilities must be attributed to persistent regenerative mechanisms. These results are also in agreement with Sechi et al [10] reported that the oil and ozone react to form ozonoid compounds that have anti-microbial properties, as well as retaining the properties of stimulating tissue regenerative pathways at a cellular level and repair for a long period.

The control group revealed minor bone regeneration after six months. The core of the regenerated interdental papilla at nine months contained disoriented periodontal ligament fibers coronal to the level of the regenerated bone. This pattern reflects repair rather than regeneration. The results found at the control group was in accordance with Nilvéus R, who reported that teeth treated with saline healed with almost complete connective tissue repair enhancing cementum formation on the root surface, increasing regeneration of alveolar bone and preventing aberrant healing events such as root resorption and ankylosis, he postulated that periodontal regenerative surgeries alone can induce mineralized and soft tissue healing . The minor regeneration capacity of the control tissues may be then attributed to the periodontal surgery. [34]

Ozone also revealed a high level of biocompatibility to fibroblasts, cementoblasts, and epithelial cells, which suggests its use against oral infectious diseases, when it comes in contact with resident oral cells, e.g. periodontal disease. [35-37]

In summary, the use of topical application of Oleozon has led to a significantly gain of clinical attachment and increased bone tissue formation restoring the lost and destructed periodontal tissues.

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