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# Evaluation of Strontium Ranelate Membrane in Gingival Recession Treatment: Histological Study in dogs.

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**Abstract: Background:** Gingival recession is the term that describes the migration of the gingival margin apical to cement-enamel junction. Strontium ranelate is a new active agent drug used for osteoporotic patients. It stimulates the new bone formation and decreases bone resorption. The aim of this study is to explore the effects of strontium ranelate membrane on gingival recession induced in dogs. **Methods:** In this study 8 adult male mongrel animal, buccal gingival recession deficiencies (upper right and left canine deficiencies in each dog) were created surgically under general anaethesia. Each GR defect was subjected to one of two therapy methods: methyl cellulose membrane with coronally advanced flap (group I control) or strontium ranelate membrane with coronally advanced flap (group II experiment). The animals were sacrificed at 8weeks with an overdose of anesthesia and the specimens were processed for histological and histometric analysis. **Results:** Histological results revealed marked regeneration in strontium ranelate membrane group compared to control group. Hestometric research showed a significant rise in the new bone and cement formation in group II instead of group I. There was also a significant decrease in amount of epithelium down growth in group II than control group. **Conclusion:** Strontium ranelate membrane is safe, inexpensive and induce regeneration in periodontal defect induced in dogs.

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## 1. Introduction

Gingival recession (GR) is oral exposure of the root surface due to a shift of the gingival margin apically [1]. It is the gingiva's most common and disturbing condition and its occurrence increases with age[2]. Localized or generalized GR is a clinical characteristic of periodontal disease and is associated with medical problems such as hypersensitivity of the root layer, abrasion of the cervical base, erosion, plaque retention, root caries, and aesthetic dis-satisfaction[3,4].

The etiology is multifactorial and involves excessive or insufficient teeth brushing, tooth malposition, damaging periodontal disease, alveolar bone dehiscence, elevated muscle attachment, aberrant frenum pull, occlusal trauma, iatrogenic factors (such as orthodontic or prosthetic therapy) and smoking [5].

GR causes loss of both soft and hard tissue, resulting in the development of numerous surgical techniques for gingival recession correction. Among the techniques reported are free gingival grafts, sliding pedicle grafts, subepithelial connective tissue grafts, envelope or tunneling techniques, the use of acellular dermal, connective tissue allografts, guided tissue regeneration and coronally advanced flap (CAF) [6,7].

The CAF is the most esthetically effective mucogingival procedure for correcting GR. It may be used to treat single or multiple sites, with adequate dimensions of keratinized gingival. In addition, there is no need for a second surgical site, as is the case with a free gingival or connective tissue graft. The results of this procedure have presented a percentage of root coverage varying from 70% to 99%, with a mean percentage of 83% [8,9].Guided tissue regeneration has become part of everyday surgical periodontal practice. These treatment modalities use barrier membranes which ignore fast growing cells (i.e., gingival epithelial, gingival fibroblasts) while enabling mesenchymal progenitor proliferation and differentiation into osteoblasts, periodontal ligament fibroblasts, and cementoblasts[10].

Different drugs were Research to achieve periodontal regeneration use local delivery. Strontium ranelate (SR) is a medication for osteoporosis which promotes bone formation by osteoblasts, unlike any other product, and inhibits osteoclasts bone resorption, as do anti-resorptive agents [11].

Strontium ranelate consists of two stable strontium atoms combined with ranelic acid that acts as a carrier [12]. Several studies have described the mechanism behind its dual mode of action by improving osteoblastic cell replication and activity and reducing differentiation and osteoclastic activity[13,14]. Methylcellulose was used A drug carrier in several pharmaceutical preparations because it is a harmless, non-toxic material that does not sensitize tissues[15]. The goal of this study was to evaluate the healing response of gingival recession induced in dogs treated with strontium ranelate membrane versus methyl cellulose membrane.

## 2. Material and the Methods:

### Materials & Gel preparation

Strontium ranelate membrane was prepared in the Faculty of Pharmacy, Phyochemistry Department follows: SR solution was prepared by dissolved 2 mg of SR (osteoStatine, Made in A.R.E,) in 2 ml distal water. Then, to make the methyl cellulose gel, seven grams of methyl cellulose powder were dissolved in 100 ml of boiled water. SR solution was then mixed into the methyl cellulose gel to make strontium ranelate gel which was sterilized at 110 ° C in the autoclave for 20 minute. A glass slab (20 cm x 20 cm) with the SR gel was also sterilized in the autoclave. The sterile SR gel and the sterile glass slab were placed in the ultraviolet incubator and the gel was spread over the glass slab and retained for 2 days in the ultraviolet incubator, which supplied a sterile atmosphere, until it dried and transformed into the membrane. This membrane sheet was sliced into several parts (2 cm x 2 cm) and placed in a ready-to-use sterile bag. To make a placebo membrane, seven grams of methyl cellulose powder were dissolved in 100 ml of boiled water then spread over the glass slab and retained for 2 days in the ultraviolet incubator until it was dry. This membrane sheet was sliced into several parts (2 cm x 2 cm) and placed in a ready-to-use sterile bag.

### Animal Selection

Eight adult male mongrel dogs weighing from 20-25 kg were selected for Surgery, Anesthesiology and Radiology Department, Faculty of veterinary Medicine, Kafr El-Sheikh University to be used in this study. The protocol of the research has been evaluated and endorsed the Animal Care Committee, Faculty of Veterinary Medicine, Kafr El-Sheikh University, under Egyptian ethical codes for experimental animal research with Egyptian ethical codes for studies on experimental animals.

### Surgical Protocol & Experimental Design

The surgical procedures were conducted under general anesthesia with premedication of Xylazine hydrochloride 2% (2.2 mg/Kg) followed by intravenous injection of Sodium Thiopental injection (8mg/kg). With 10% povidine-iodine solution 1% titrable iodine, the surgical sites were disinfected. Using a tiny round bur (N1), a notch was put at the gingival margin. Miller class 1 buccal gingival recession defects (upper right and left canine defects in each dog) have been developed surgically. Two vertical incisions were produced from the gingival margin and expanded 5 mm apically on the buccal surfaces of the canines. A horizontal incision linked these vertical incisions apically[16].

The gingival tissue limited by the 2 incisions was removed using a periosteal elevator. Using low speed rotary burs with sterile saline irrigation, the exposed bone was removed. At the end of the surgery, an apical knot was positioned using a tiny round bur as a landmark for histopathological examination (N2) (Fig. 1a). In order to produce plaque infected gingival recession defects, all experimental sites were permitted to accumulate plaque for a period of 4 weeks (Fig. 1b). After four weeks, one of the following treatments was randomly allocated to the defects:

**1-** Methyl cellulose membrane with coronally advanced flap (placebo) (control group I).
**2-** Strontium ranelate membrane with coronally advanced flap (experimental group II).

Two oblique incisions were developed in conjunction with an intrasulcular incision. After the MGJ, a complete thickness trapezoidal mucoperiosteal flap has been lifted. Curettes were applied to the root surface and the necrotic cement layer was removed. The root surface was conditioned for 4 minutes using the cotton pellet burning method with a saturated tetracycline hydrochloride solution (250 mg/ml) and altered at intervals of 30 seconds. The root surface was rinsed with sterile saline for 1 minute after treatment. After root surface conditioning, SR membrane was adjusted to cover the deficiency and the flap was coronally developed to cover the membrane and sutured using easy interrupted sutures (Fig. 2 a,b,c).

### Postsurgical Care:

- Dogs got Acupan i.m every 12 hours for pain control for 2 days following surgical processes.
- Intra-muscular administration of antibiotics, tetracycline HCL (Terramycine retard 125 mg i.m., every 12 hours) during the first 2 days post-operatively. Next, a tetracycline HCL capsule (250 mg) was mixed with the dog's food (three times per day) for 7 days.
- Postoperative plaque control was conducted daily by 0.12% chlorhexidine gluconate irrigation.
- During the study assessment period, dogs were fed a smooth diet to decrease the chance of mechanical interference with food consumption.

**Histopathologic Examination:**

- After 8 weeks, an overdose of anesthesia was used to sacrifice the animals for histological examinations. The jaws were dissected and the experimental specimen blocks (canine segment) were acquired. For one week, the blocks were fixed in a 10% neutral formalin solution.
- Until demineralization happened, blocks of biopsy samples were transmitted to 10% EDTA solution containing 5% sodium sulfide. The decalcified specimens were washed in running water, dehydrated and embedded in paraffin wax (the wax melting point of paraffin should not exceed 56 ° c to prevent tissue burning) for 24 hours and then embedded in blocks of paraffin. All tissues embedded in formalin-fixed-paraffin were serially sliced bucco-lingual with a 5 µm thick rotary microtome. The parts were then placed on glass slides and overnight incubated at 60 ° C after which they were rehydrated into xylene, rinsed in ethanol and then water. The slides were stained with hematoxyl stain and counterstained with eosin. In the histological examination, the 2 notches mentioned above were used as reference points.

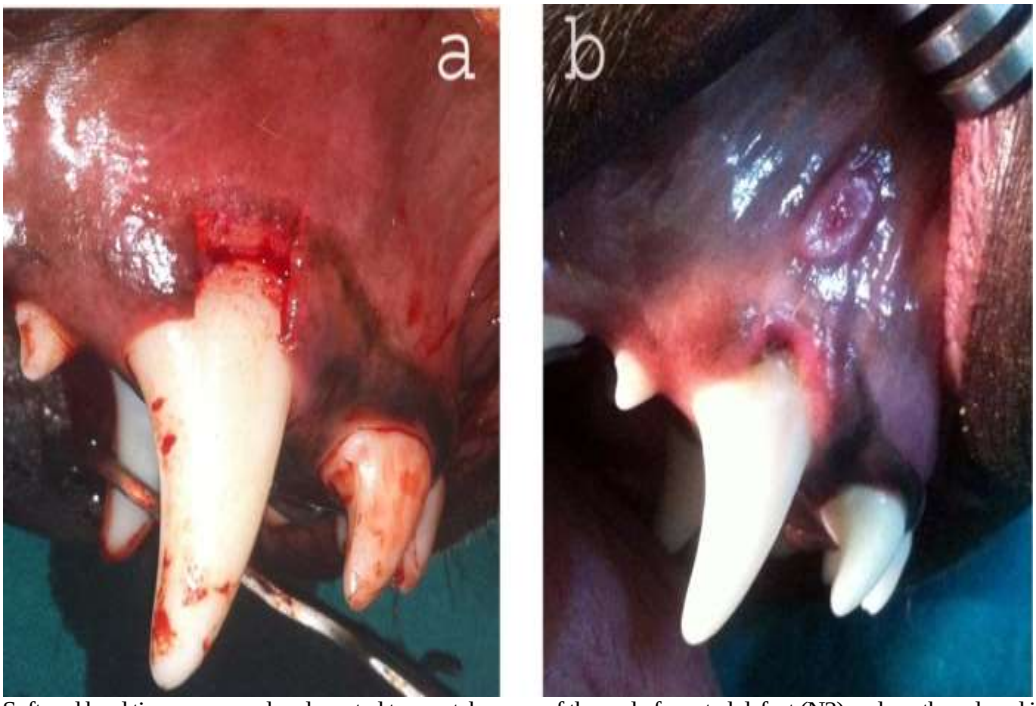



Fig. 1: a, Soft and hard tissue removed and created two notches one of the end of created defect (N2) and another placed in the gingival margin preoperative position (N1). b, Created gingival defect after 1 month of plaque accumulation.


Fig. 2: a, Reflection of full thickness flap. b, Membrane adaptation in the defect. c, The flap coronally advanced to complete cover of strontium ranelate membrane and suture.

**Histometric Assessment**

Histological sections were photographed and digitized with a light microscope coupled by a digital camera, at a lenses magnification of 100x that connected to a monitor and a personal computer using the crest of the alveolar bone and two notch as reference point. Thus, linear measurements were performed. The following variables were analyzed:

• **Epithelial length (EL):** distance from the gingival margin to the most apically located epithelial cells.
• **Connective tissue adhesion (CT):** distance from the apically extension of the junction epithelium to the coronal extension of the new cementum.
• **New cementum formation (NC):** distance between the apical border of the reference notch and the most coronal extent of cementum-like tissue.
• **New bone formation (NB):** measured from the apical border of the reference notch to the most coronal extent of bonelike tissue.
• **Defect extent (DE):** distance between the apical notch and the coronal notch.

**Statistical Analysis:**

Statistical analysis was performed using SPSS version 21 (SPSS Inc., Chicago, IL, USA). For statistical evaluations, the groups were evaluated using the independent sample t test P values <0.05 were considered statistically significant.

**3. Results:**

**1- Clinical results:**

Clinically, the healing response was favorable for all treatments. SR and methyl cellulose was well tolerated by the periodontal tissues of all animals that showed no signs of inflammation. The post-operative edema was slight and gradually disappeared. Also, no adverse reactions such as allergies or abscesses were observed in any of the animals.

**2- Histological Observations: Light microscopic results (H &E Staining):**

**Control group:**

At the end of the experiment, group I (control group) demonstrated epithelial growth down on the root surface, loose connective fibrous tissue is formed with little bone formation (Fig. 3 a,b). Another section of control group showed bone regeneration along the alveolar bone, the new bone demonstrated few entrapped osteocytes within lacunae. Poor-oriented periodontal fibers were observed and the absence of typical structure of Sharpey's fibres extending between the new cementum and the newly formed bone. The new bone demonstrated few entrapped osteocytes within lacunae. Dilated blood vessels also observed (Fig. 4 a,b,c).

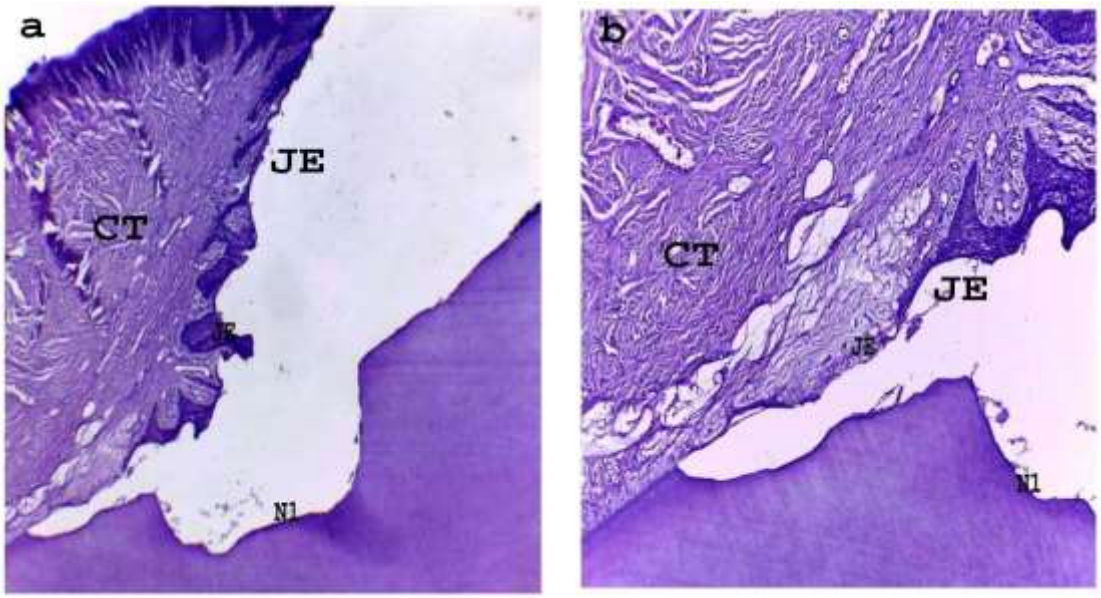



Fig. (3): Photomicrograph of a group I (control group): Labiolingual section of methyl cellulose membrane treated gingival recession defect showed. a) Epithelial growth down along the root surface (EDG), loose connective fibrous tissue is formed (CT) (H&E .OMX40). b) High power of epithelial down growth (EDG), JE (Junctional Epithelium), N1 (reference notches) (H&E, OMX100).

**Experimental Group (Strontium Ranelate Membrane):**

At the end of the experiment, group II (Experimental group) showed complete healing of the periodontium that demonstrated similar features to native periodontium. Woven bone and osteoid tissue are seen adjacent to the newly regenerated bone. The periodontal ligament well oriented, extended between the new cementum and the newly formed bone (Fig. 5 a,b). A larger amount of periodontal ligament (PDL) and typical structure of sharpey's fibres were regenerated and increased blood vessels. New cementum grew prominently along the root surface in the strontium treated group compared to the control treated group. More and thicker bone trabeculae are formed with multiple reversal lines (Fig. 5 c,d).

At OMX200 magnification, section of strontium ranelate group revealed marked bone deposition with numerous osteocytes entrapped within the new bone formed and significantly higher osteoblast numbers were detected at the periphery of recently formed bone, newly regenerated functionally oriented vascular PDL groups of fibers were observed only in the experimental group (Fig. 6 a,b).

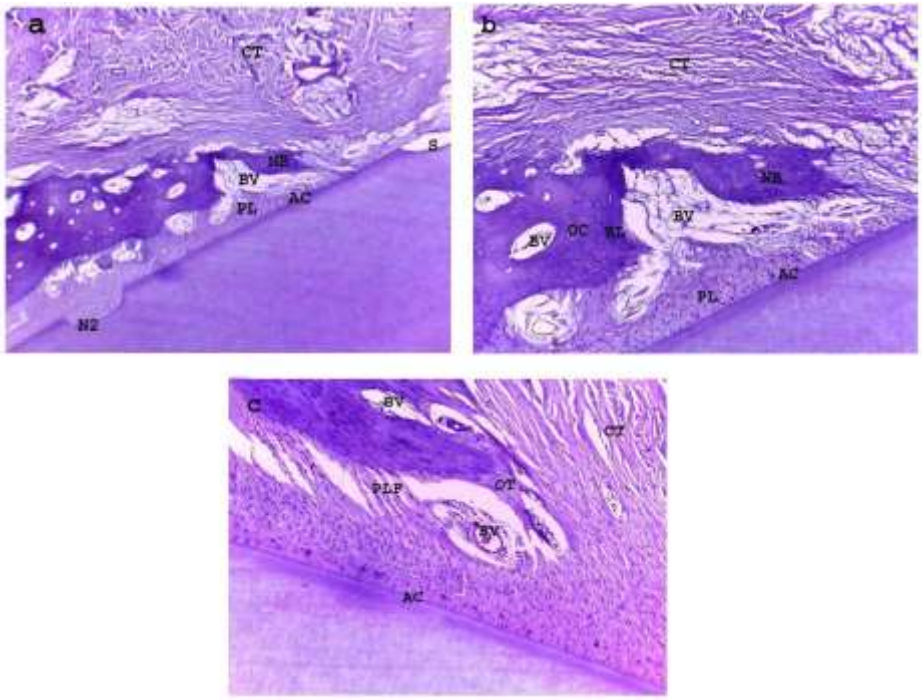



Fig. (4): Another histopathological section of the control group showed, a) new bone (NB) reaching coronal to the notch area ( N2), loose connective fibrous tissue is formed (CT) (H&E, OMX100). b) New bone (NB), with reversal lines (RL) and osteocytes (OC). Poor-oriented periodontal fibers (PL), dilated blood vessels (BV) and cementum formation (NC) (H&E, OMX200). c) Absence of typical structure of Sharpey's fibres (PLF) (H&E, OMX400).

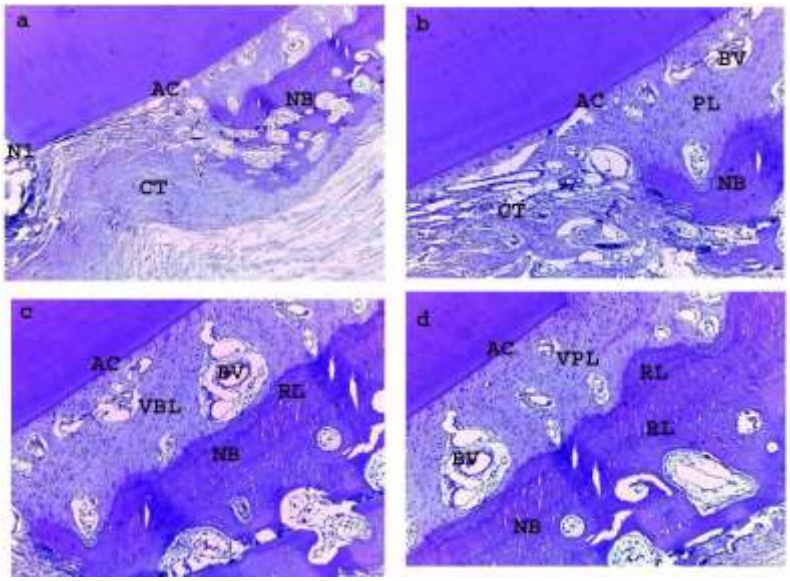



Fig. (5): Photomicrograph of a group II (Experimental group). a) thick connevtive tissue (CT), marked newly Bone formation (NB) and newly formed acellular cementum (AC) (H&E, OMX100). b), marked Newly Bone formation (NB), a layer of acellular cementum over the defect, functionally oriented periodontal ligament fiber (PL). Area of woven bone and osteoid tissue also observed (black arrows) (H&E OMX200). c), d) typical structure of Sharpey's fibres were regenerated (VPL) and increased dilated blood vessels (BV). Increased reversal lines (RL) within the bone (H&E OMX200).

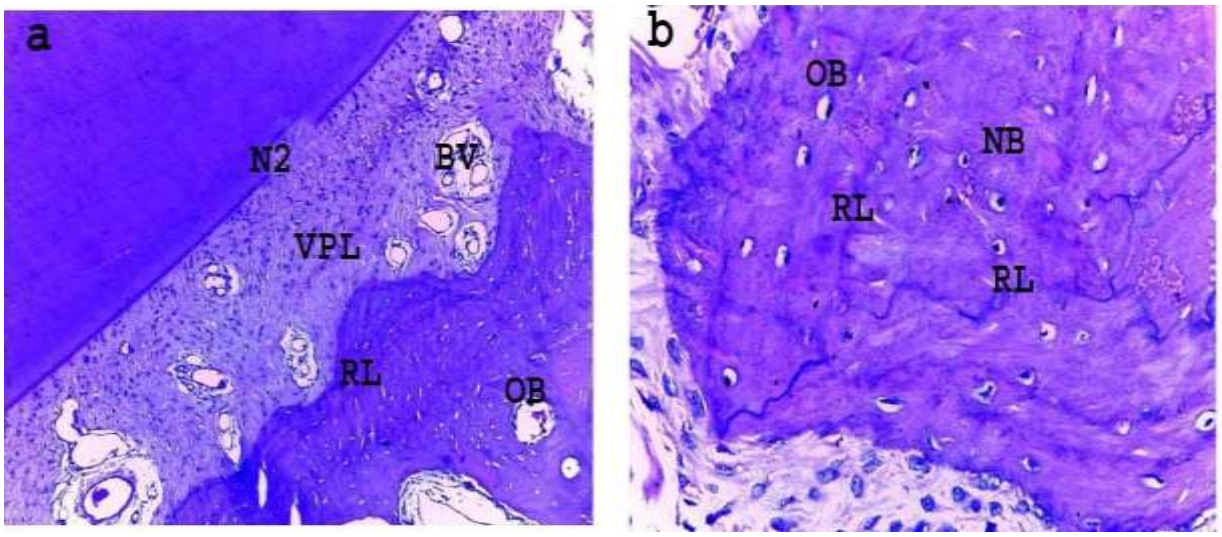



Fig. (6): Another histopathological section of experimental group presented a) marked newly bone formation (NB) above the notch (N2), and cementum formation (NC). Functionally oriented periodontal ligament fiber (VPL), dailated blood vessels (BV) (H&E, OMX200). b) Higher magnification of the previous image showedmarked bone deposition with numerous osteocytes entrapped within the new bone (OC), increased reversal lines, and marked peripheral osteoblastic activityat the periphery (Red arrows) (H&E, OMX400).

**3-Histometric Measurement:**

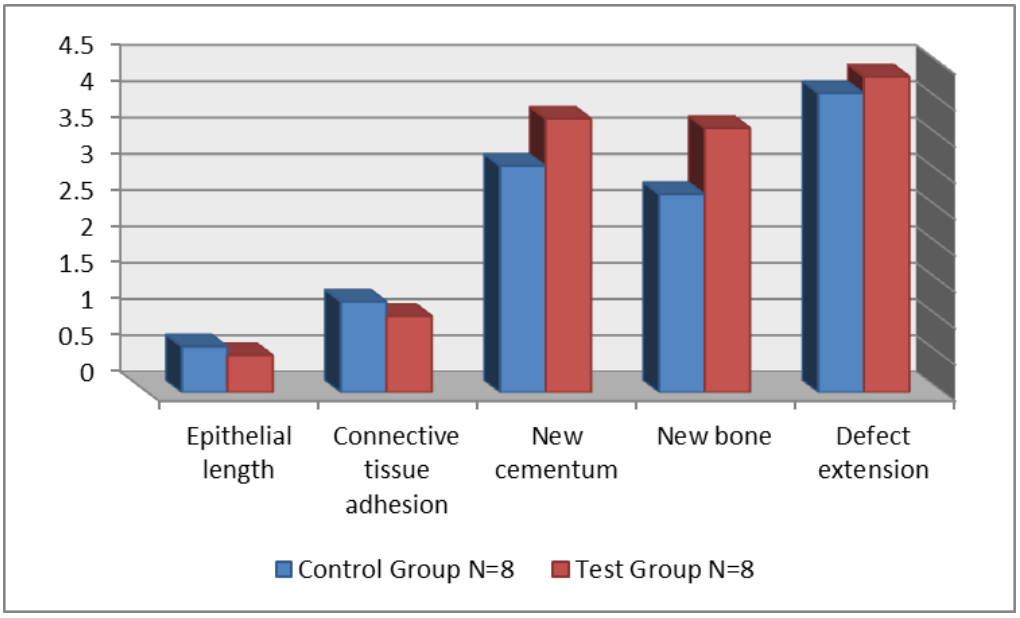
The histometric results of EL, CT, NC, NB and DE are given in Table (1), graph (1). Data analysis showed major differences between the two treatment groups regarding the initial defect height (4.12±0.23, 4.35±0.37 for control group & test group respectively) (P= 0.169).

Inter group analysis demonstrated that test group showed a superior and significant height of new bone and cementum extension (3.64± 0.34, 3.77± 0.23 respectively) than the control group (2.73±0.28, 3.12± 0.35 respectively) (P<0.01, P< 0.001,).

Additionally, data analysis demonstrated insignificant differences between the treatment modalities in the connective tissue adhesion along the root surface (1.25± 0.19, 1.05±0.16, for group 1 &2 respectively) P>0.05 (Table 1). In addition, t-test showed Difference statistically significant between the treated groups in the epithelial length (0.63 ± 0.1, 0.51 ± 0.06 for group 1 &2 respectively) (P>0.05).

Table (1) Histometric analysis of the measured parameters in control groups and test group (mean± standard deviation in mm).

| Tested Parameter | Control Group N=8 Mean ± SD | Test Group N=8 Mean ± SD | t-test P-value |
|---|---|---|---|
| Epithelial length | 0.63 ± 0.1 | 0.51±0.06 | 2.620 0.021* |
| Connective tissue adhesion | 1.25± 0.19 | 1.05±0.16 | 2.080 0.058 |
| New cementum | 3.12± 0.35 | 3.77± 0.23 | 4.095 0.001** |
| New bone | 2.73±0.28 | 3.64± 0.34 | 5.628 0.000*** |
| Defect extension | 4.12±0.23 | 4.35±0.37 | 1.457 0.169 |




Graph (1): Column chart representing mean of the quantitative measurement of the difference between the means of parameters between groups in mm.

**4. Discussion:**

For many years, treatment of gingival recession has presented therapeutic challenge for clinicians. Numerous plastic surgical procedures for root protection have been suggested to minimize root resistance, enhance esthetics and treat defects arising from root caries and/or cervical abrasions [17].

In the present study, tetracycline was used for root conditioning to remove the smear layer and expose the openings of dentinal tubules which would enhance the regenerative potential of the periodontium. All of the selected mucogingival techniques were similar in type of defect, root conditioning, flap design, positioning of membranes, and suturing technique. Coronally advanced flap (CAF) with vertical releasing incisions was used in the current study to increase the predictability of achieving adequate coronal positioning and complete defect coverage.

Strontium ranelate was researched in different models of rats, including intact bodies, osteopenia induced by immobilization and osteoporosis induced by ovariectomy, Strontium ranelates increased bone formation markers and decreased bone resorption markers in these in vivo experiments, promoting bone gain as reflected in increased external diameter of long bones. These beneficial impacts on the bone were achieved without influencing bone mineralization [18]. Therapy with strontium ranelate was predictive enhanced bone strength, which was indirectly confirmed by the decrease in the danger of fracture in clinical trials[19].

In this analysis, during the experimental length, no suppuration or abscess formation was observed in both classes. Such results are in line with Group 2 (test group) Nunes et al.,[20] and Pelletier et al., [21]who found that SR has anti-inflammatory effect by reduced release of cytokines tumor necrosis factor alpha (TNF-α) and interleukin 1 beta(IL-1β).

In the current study healing of the recession defects was assessed after two months in dogs to evaluate and compare the regenerative response induced by the tested materials, SR membrane versus methyl cellulose membrane. Periodontal bone regeneration can be evaluated histologically after 8-week healing interval [22,23]. Also Choi et al., [24] showed that, no significant difference was observed between bone regeneration at 8 and 24-weeks interval. Therefore, in the present study, 8-weeks healing period were enough to observe the wound healing process.

The ultimate goal in periodontal therapy is regeneration. The establishment of a new connective tissue attachment with fibers inserting into new bone and new cementum on a previously diseased root surface is the ideal objective.

Histological evaluation remains the only reliable method to determine the efficacy of periodontal therapies[25], so we choice the histologically and histomorphometrically analysis for evaluation the effect of SR membrane or methyl cellulose membrane on the healing of surgically created Miller class I gingival recession in dogs. The placement of 2 reference notches was helpful, as N1 (the gingival notch) represented the exact location of the pre-operative gingival margin and N2 (the bone notch) represented the end of the defect. Consequently, when any connective tissue attachment was observed in postsurgical examination coronal to N2, it was considered as new periodontal regeneration.

At two months, in group 1, overall, the histological analysis of specimens revealed bundles of loose connective fibrous tissue with little bone formation. This bone formation may be attributed to action of methyl cellulose membrane which acts as guided tissue regeneration excluding fast growing cells (i.e., gingival epithelial, gingival fibroblasts) while enabling mesenchymal progenitor proliferation and differentiation into osteoblasts, periodontal ligament fibroblasts, and cementoblasts[10].

At 2 months in the current study, in test group periodontal ligaments were found to be very dense and well organized highly cellular component that were inserted into the newly formed layer of cementum and new bone. Newly formed bone and osteoblastic cells were found. No migration of junctional epithelium was observed in test groups.

New bone formation was demonstrated by Nahass et al., [26] who concluded that local application of SR could up-regulate the bone formation and may prove to be a cost-effective method of bone regeneration. Strontium ranelate significantly increased alkanin phosphate (ALP) activity and osteocalcin (OCN) mRNA expression that induce bone mineralization. SR has the ability to stimulate cyclooxygenase2 (COX2) expressions, COX2 promoter activity, and prostaglandin E2 (PGE2) production which induce osteoblastic differentiation in marrow stromal cells, Furthermore, SR Promotes osteoblast replication which could justify the marked prevalence of osteoblasts in SR groups[27].

Our results were in accordance with Cao et al., [28], who state that insulin alone and combined with SR were both effective in accelerating bone fracture healing their combined treatment showed significant improvement in promoting osteogenic marker expression compared with insulin alone. This result in agree with Amaral et al., [29] who concluded that bone formation process seemed to intensify of SR. Dual mechanism of action can be linked to the formation of new bone in SR unit, favoring bone metabolism through inhibition of osteoclasts and stimulation of osteoblasts.

Histomorphometric findings showed that SR treated group showed an excellent healing that exhibit new bone, cementum and functionally oriented periodontal ligament as compared to the control group. These findings are in agree with Elgendy & Shoukheba[30], who strontium ranelate 2% gel appears to be safe and may support periodontal wound healing/regeneration in intrabony periodontal defects without complications.

Bone biomechanical and structural characteristics such as mineral density can be increased by SR. It connects crystal surfaces like calcium to hydroxyapatite and plays a critical part in the process of bone mineralization [31,32]. There are two possible action mechanisms described in SR literature: 1) activating calcium-sensing receptor (CaSR) or cation-sensing receptor; and 2) growing osteoprotegerin (OPG) expression in relation to reducing osteoblast receptor activator of NFκB ligand (RANKL) expression [33].

The CaSR is critical for bone development, and specific effects of strontium are mediated by the CaSR. Increases in bone cell replication caused by strtrontium ranelate are CaSR-dependent. Strontium ranelate has pro-apoptotic effects in bone resorbing osteoclasts via the CaSR[34]and in osteoblasts, strontium ranelate-induced stimulation of replication byCaSR[35].

Finally, receptor activator of nuclear factor kappa-B ligand (RANKL), receptor activator of nuclear factor kappa-B (RANK) and osteoprotegerin (OPG) would have extensive functions beyond regulation of bone remodeling. RANKL/RANK Signaling controls the formation, stimulation and survival of osteoclasts in normal bone modeling and remodeling and in a range of pathological conditions with increased bone turnover. Through binding to RANKL and preventing it from binding to RANK, OPG prevents bone from unnecessary resorption and preventing it from binding to RANK. Strontium ranelate induced increases in mRNA levels of OPG and decreased RANKL mRNA [36].

**Conclusion**

In conclusion, the current study showed that bone formation could be up-regulated by regional application of SR. Regional use of SR can be a cost-effective and safe tool for stimulating bone formation and treatment of gingival recession.

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