



## The Effect of local injection of strontium on inhibition and repair of orthodontically induced root resorption in rats (An experimental study)

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### Abstract

**Background:** Strontium (Sr) is an alkaline earth trace metal cation that has a high affinity for hydroxyapatite. This study aimed to examine the histological effect of locally injected strontium (Sr) on prevention and repair of induced root resorption of rat molar subjected to orthodontic tooth movement.

**Materials and Methods:** Eighteen ten-week- old male Wister rats were randomly divided into two groups of nine animals for two experiments. In both experiments; uniform standardized expansive springs were used to move maxillary first molars buccally for three weeks and then removed. In experiment No.1, at the time of spring placement, 0.25 ml of the strontium chloride solution at a concentration 240 mg/ml was injected at the sub-periosteum of the buccal side of the maxillary left first molar which was the experimental side. On other hand, 0.25 ml of distilled water was injected at the sub-periosteum of the buccal side of the right maxillary first molar which was the control side. The injections repeated every other day for three weeks whereas in experiment 2 it was begun at the day of spring removal and repeated every other day for another three weeks. After that the animals were scarified humanly and biopsies were taken for histological examination.

**Results:** In experiment 1 the side injected with strontium showed statistically significant lesser root resorption than control side, while in experiment two the strontium injected side showed significantly more repair.

**Conclusion:** Results of the study suggested that local injection of strontium can inhibit root resorption process and enhance repair.

**Keywords:** Strontium, inhibition, repair, root resorption.

### Introduction

Root resorption is defined as destructive process which leads to a subsequent progressive or transitory

loss of cementum or cementum/dentin. This process may be physiological or pathological. Root

resorption occurrence may be caused by dental trauma and chronic inflammatory process of periodontal tissue; also, root resorption may be induced by tooth eruption, impacted teeth, by the pressure exert by extreme orthodontic tooth movement and by tooth implantation or reimplantation<sup>(1-3)</sup>. Root resorption mainly occurs due to the action of activated clasts (Figure1). Due to the similar morphology, enzymatic properties and function of the cells causing dentin, cement and bone resorption, the processes of root and bone resorption may be considered similar. Thus, drugs or other substances that are able to inhibit bone resorption may also be effective for treating root resorption<sup>(3,4)</sup>.

Potential effects of several pharmacological agents on orthodontically induced root resorption have been examined in several experimental studies and some of these agents have been applied clinically. The inhibitory effects of bisphosphonates like (AHBuBP)<sup>(5)</sup>, risedronate<sup>(4)</sup> and clodeonate<sup>(6)</sup> which putatively interfere with the function of clast cells and, hence, inhibit both bone and root resorption, have been shown. Doxycycline has been shown to reduce the total number of osteoclasts and prevent root resorption<sup>(7-9)</sup>. Treatment modalities, based on targeting osteoclasts, could have been used in clinical orthodontics<sup>(10,11)</sup>.

In a recent study by AL\_Duliamy<sup>(12)</sup>, it was shown that local injection of strontium significantly reduced the rate of experimental and relapsed tooth movement in rat. These results suggest that local injection of strontium could be useful in enhancing anchorage or retaining teeth in orthodontic treatment. In that study, local injection of strontium inhibited alveolar bone resorption and suggested

that the formation and/or viability of osteoclasts were affected by strontium.

According to Kim et al.<sup>(13)</sup>; Tyrovola et al.<sup>(14)</sup>; Seifi and Jessri<sup>(15)</sup>; and Hamman<sup>(16)</sup> the RANKL/OPG balancing system is important in regulating RANKL and OPG in periodontal tissues and for osteoclastogenesis and is therefore critical to regulate bone remodeling during orthodontic tooth movement as well as root resorption.

Boyle et al.<sup>(17)</sup> proposed that variations in RANKL and OPG expression play an important role in root resorption. The ability of strontium to modulate these cytokines suggests it is able to down-regulate osteoclastogenesis through an indirect stimulation of osteoblasts<sup>(18)</sup>.

Odontoclasts and cementoblasts share similar regulatory pathways with osteoblasts and osteoclasts<sup>(19)</sup>, therefore a study to examine the effect of strontium in the inhibition of root resorption which induced by orthodontic tooth movements was suggested. In the present study, the effect of local injection of strontium on both root resorption during experimental tooth movement and on the repair process after experimental tooth movement in rats was examined histologically.

## Materials and Methods

Eighteen ten-week- old male Wister rats weighing 250- 300 g were purchased from the animal house of the National Center of Drug Control and Research/ Baghdad- Iraq and used as experimental animals. The animals were treated ethically according to the guidelines of the Animal Care staff at the National Center for Drug Control and Research / Baghdad-Iraq. Strontium chloride was the kind gift from the Chemistry Department of the College of Science at AL-Mustansirya

University. Concentration of strontium chloride was extrapolated from doses recommended by Ammann et al.<sup>(20)</sup>. The experiments involving tooth movement were performed according to the methods used in a previous study by AL\_Duliamy<sup>(12)</sup>.

The rats were divided equally to conduct two separate experiments, one to study the amount of root resorption that occurred during three weeks of tooth movement and another study to evaluate the amount of repair of the resorption areas that happened after stopping tooth movement for another three weeks.

In both experiments, uniform standardized expansive springs were used for moving the maxillary first molars buccally (Figure2A). The spring initially generated an average expansive force of 20 g on each side, and it exerted as continuous force of intermediate magnitude for molars expansion (Figure2B). In experiment 1, at the day of spring placement, 0.25 ml of the strontium chloride solution at a concentration 240 mg/ml was injected under general anesthesia by an extra-fine needle syringe locally at the sub-periosteum of the buccal side of the maxillary left first molar which was the experimental side. On other hand, 0.25 ml of distilled water was injected at the sub-periosteum of the buccal side of the right maxillary first molar which was the control side. The injection repeated every other day under general anesthesia for three weeks. At the end of the third week of force application, the springs were removed, the animals were scarified humanly and biopsies were taken for histological examination. In experiment 2, the springs were left for three weeks, without any injection, to move the maxillary first molars buccally, then they were removed and injection started using the same regimen as in experiment 1 and

repeated every other day for another three weeks. After that animals were scarified and biopsies were taken for histological examination.

### Histology

After the humanly scarification of animals under general anesthesia, each maxilla was dissected into two halves (experimental and control) each half including the three maxillary molars, according to Igarashi et al.<sup>(4)</sup> The specimens were then dehydrated and embedded in paraffin. Each specimen was cut at a coronal level into serial 5 $\mu$ m -thick cross-sections with a microtome. The sections were stained with hematoxylin and eosin and observed with a digital light microscope. The mesio-buccal root (Figure3) of the experimental and control maxillary first molar was identified and examined. The root was divided into buccal and palatal sides, based on the mesio-distal axis of the root. Evaluation of the root resorption areas was performed on the root surface of the pressure side of both control and experimental sides. The root-resorption area was measured at  $\times 40$  magnification by the inbuilt image processing software of digital microscope (Micros Crocus II MCX100LCD Produktions und HandelsgmbH) that was fed directly to a TV monitor with a real time live camera, according to a method described previously<sup>(4)</sup>. The size of the root-resorption area was expressed as  $\mu\text{m}^2$  (Figure4). Values for four sections, selected at five section intervals, were averaged for each animal.

### Statistics analyses

The data from the control and experimental sides of both experiments were compared and statistically analyzed by Paired t-test at 5%

significance level using SPSS software.

## Results

**Experiment 1:** From observations on Table (1) and Figure (5) it was found that the mean value of the size of root resorption areas in the buccal side of the mesio-buccal roots of the right maxillary first molars (control side) was statistically significantly higher than the size of resorption areas found in the buccal side of the mesio-buccal roots of the left maxillary first molars (experimental side) ( $P < 0.001$ ).

**Experiment 2:** From observations on Table (2) and Figure (6) it was found that the mean value of the size of repaired areas was significantly higher in the buccal side of the mesio-buccal roots of the left maxillary first molars (experimental side) Figure (7) than the size of the repaired area found in the buccal side of the mesio-buccal roots of the right maxillary first molars (control side) ( $P < 0.001$ ).

## Discussion

The coronal level of the roots undergoes the greatest change after force application<sup>(21-24)</sup>, therefore, for each maxillary first molar (experimental or control), the percentage of root resorption was measured at the coronal area of each mesio-buccal root.

### √Inhibitory effect of strontium on root resorption

Root resorption occurrence in the experimental sides of this study was

significantly lower than that in the control sides. This emphasizes the local injection of strontium characteristics in limiting the resorptive processes of root. No experimental data are available on the effect of strontium on root resorption to compare with this study. The lesser occurrence of root resorption in the experimental sides can be explained by the action mechanism of strontium through its ability to modulate RANKL/OPG balancing system<sup>(25)</sup> which is important in regulating RANKL and OPG in periodontal tissues and is therefore critical to regulate root resorption during orthodontic tooth movement<sup>(16,26)</sup>.

### √Effect of strontium on the repair of root-resorptive lacunae

After three weeks of tooth movement, extensive root resorption (Figure 8) had occurred on the buccal side of the mesio-buccal roots of both the right and left maxillary first molars. Most studies on root resorption showed that the repair processes did not start until the applied force was released<sup>(4,27,28)</sup>. Therefore after three weeks of force application the springs were removed and local injection of strontium started and continued for another three weeks to allow repair processes to take place. The significant differences in the sizes of repaired lacunae between control and experimental sides indicated that the local injection of strontium enhance the reparative processes of the root. This may be explained by the action of strontium as it has been one of the leading trace elements applied to induce remineralization of dentinal tubules<sup>(29,30)</sup>.

## Clinical Relevance

External root resorption is a frequent adverse effect of orthodontic tooth movement. Root resorption of

permanent teeth caused by orthodontic tooth movement, implantation or replantation of teeth is also an unsolved problem in dentistry. This root resorption can impair successful treatment and, in certain situations, may decrease the functional capacity or the actual life of the tooth. So, if root resorption can be prevented by pharmacological agents, this complex problem may be overcome. Therefore, it would be of clinical significance for this study to demonstrate for the first time that root resorption incident to orthodontic tooth movement could be prevented and repaired by local injection of strontium and further study must be undertaken to confirm the exact mechanism of using strontium in human. This result would be an important contribution toward reducing risk factors in orthodontic treatment.

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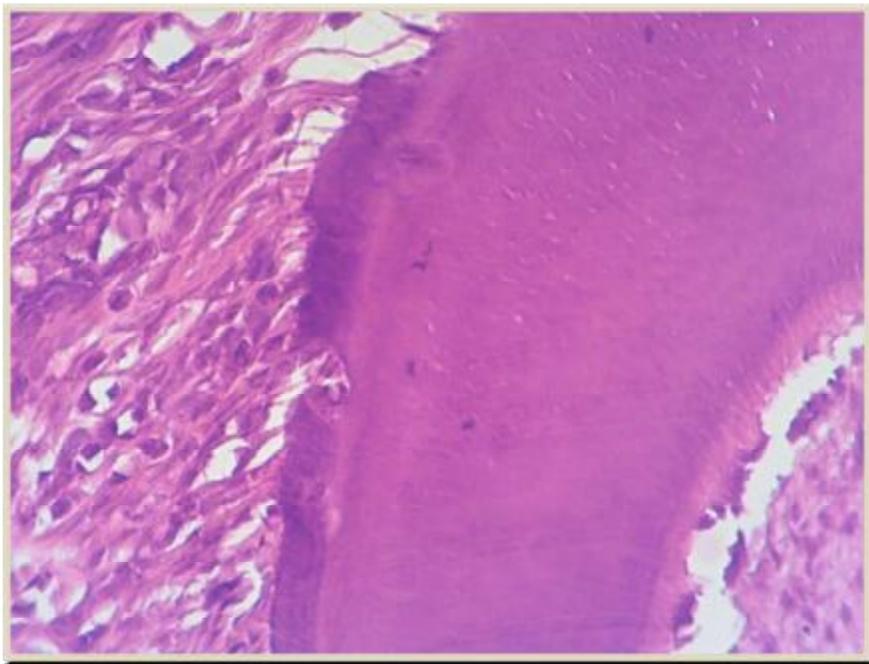
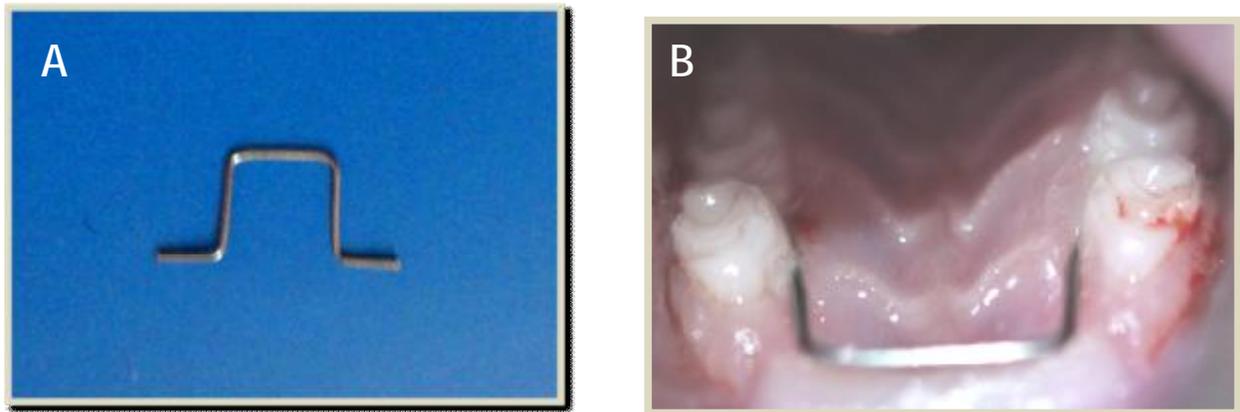
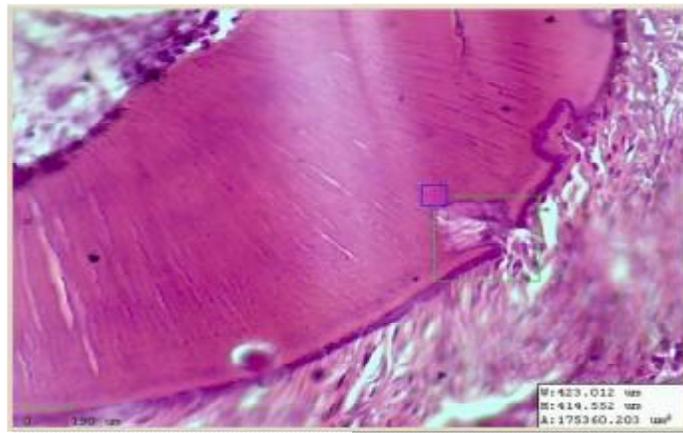


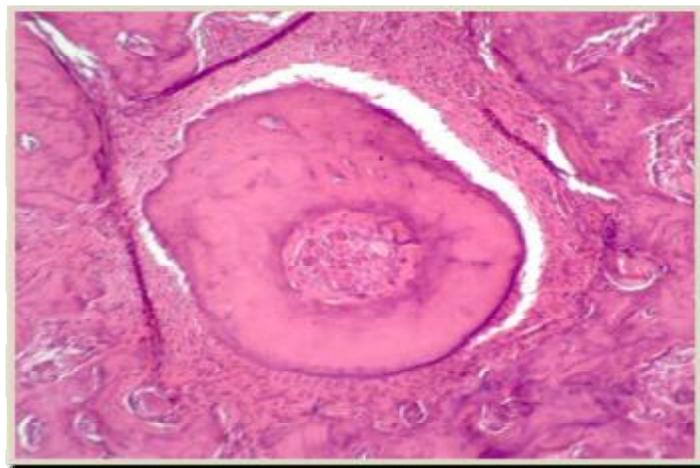
Figure 1: Clast cell caused root resorption area. (H & E Staining × 40)



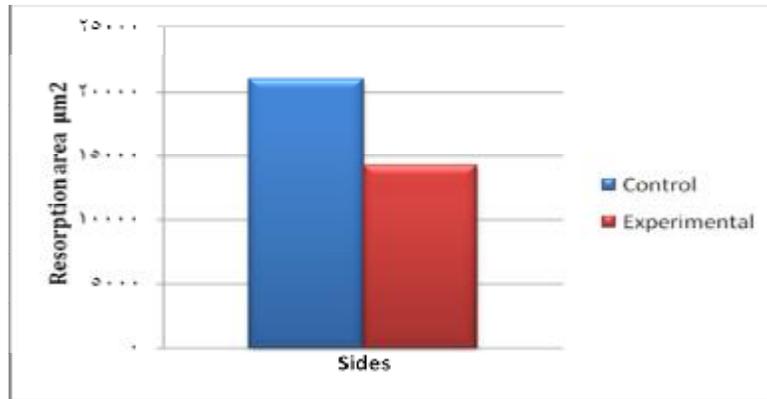
**Figure 2:** (A) The spring , (B)The spring in its place in rat maxilla.



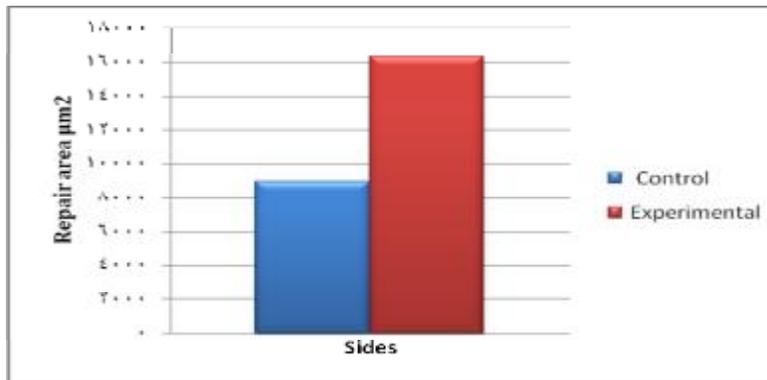
**Figure 3:** Pressure and Tension sides of the mesio-buccal root of the maxillary first molar in experimental side. (H & E Staining  $\times 4$ ).



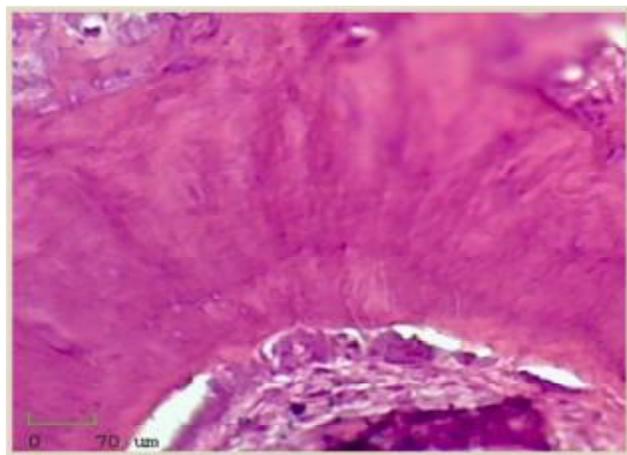
**Figure 4:** The size of the root-resorption area expressed as  $\mu\text{m}^2$ . (H & E Staining  $\times 40$ )



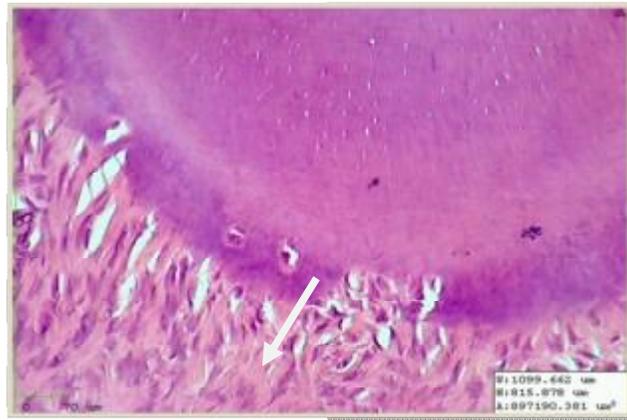
**Figure 5:** Bar Graph represents Mean root resorption area on the buccal side (pressure) of the mesio-buccal root of the maxillary first molars of the Control and Experimental Sides in experiment 1



**Figure 6:** Bar Graph represent Mean repair area on the buccal side of the mesio-buccal root of the maxillary first molars of the Control and Experimental Sides in experiment 2



**Figure 7:** Repair of the resorbed root surface on the buccal side of the mesio-buccal root of the upper first molar in Experiment 2 (experimental side). Arrowhead indicates the apposition of repair cementum (cementoid) in the root-resorptive lacunae.



**Figure 8:** Extensive root resorption on the buccal side of the mesiobuccal roots of the upper first molar (Arrowhead) at the end of the third week in the control side in experiment 2. (H & E Staining  $\times 40$ )

**Table 1:** Descriptive Information of the resorption areas size and the t - test for Experimental– Control Difference in experiment 1

P. Value	Variables					Sides
	SE.	SD.	Mean.	Max.	Min.	
.000*	49.503	148.511	21122.666	21405	21000	Control side
	29.899	89.699	14329.777	14511	14210	Experimental side

N = 9, df= 8, \*= Highly significant difference

**Table 2:** Descriptive Information of repair areas size and the t - test for Experimental– Control Difference in experiment 2

P. Value	Variables					Sides
	SE.	SD.	Mean.	Max.	Min.	
.000*	65.473	196.419	9125.777	9500	9000	Control side
	49.403	148.209	16488.000	16770	16330	Experimental side

N = 9, df= 8, \*= Highly significant difference