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Tissue response to space closure in monkeys: a comparison of orthodontic magnets and superelastic coil springs

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SUMMARY Interest in using magnets for generating orthodontic forces started with the widespread availability of rare earth magnetic alloys. *In vivo* studies have indicated that a static magnetic field and/or corrosion products from the magnetic materials may induce biological effects when in close contact with cells or tissues. In the clinical situation, orthodontic magnets are often situated some distance away from the gingiva and bone. Consequently, the previously observed biological effects may not be found in an experimental situation mimicking the clinical setting. Thus, the present experimental study was undertaken to test this hypothesis using commercially available cobalt-samarium magnets for orthodontic treatment in comparison to treatment with Sentalloy closed coil springs with respect to possible side effects on alveolar bone growth, gingival epithelial thickness as well as rate of space closure.

Corrosion of the uncovered areas of the magnets was already evident after 6 weeks. No statistical differences were found between the magnet and coil spring specimens with respect to rate of space closure, bone formation or epithelial thickness. The only two variables that differed significantly between magnet and coil spring specimens was that there were more resorption and more tetracycline labelled osteocyte lacunae under the magnets. In conclusion, although some marginal statistical differences were found between the magnet and coil spring specimens with respect to cell and tissue reactions, the near lack of cell and tissue effects of the magnets in the present clinical experimental situation compared to previous studies in which the magnets were positioned in close contact with the tissue under study, indicate limited adverse clinical effects.

Introduction

Interest in using magnets for generating orthodontic forces started with the widespread availability of rare earth magnetic alloys (Becker, 1970; Blechman and Smiley, 1978). Rare earth magnets, initially made from cobalt-samarium (Co_5Sm) alloy and later neodymium-iron-boron ($Nd_2Fe_{14}B$) alloy generate high mechanical forces in relation to their small size. With this type of device, orthodontic treatment with magnets has become a practical proposition in certain applications (Sandler *et al.*, 1989). However, paired magnets are inevitably surrounded by a magnetic field.

Magnetic fields have experimentally been shown to be able to provoke biological responses (Becker, 1963; Barnothy, 1964, 1969; Busby, 1968; Roth, 1968). A series of studies on the biological effects of orthodontic Co_5Sm magnets have shown that the magnetic field and/or corrosion products from the magnetic material itself are not biologically inactive. Linder-Aronson and Lindskog (1991) showed that permanent Co_5Sm magnets applied in close contact with the rat hind leg resulted in increased bone resorption of the tibia and a thinner epithelium after 4 weeks. Using the same experimental method Linder-Aronson and Rygh (1994) found a reduced cortical and total bone width in areas of the tibia in close contact with permanent Co_5Sm magnets. Similar magnets in close contact with oral epithelium on the alveolar processes in monkeys were found to provoke a reduction in epithelial thickness and tetracycline (TC) uptake in the bone underlying the magnets (Linder-Aronson *et al.*, 1992). It could, however, not be determined whether these effects were due to the magnetic field or corrosion products from the magnets. Furthermore, the study was hampered by a limited number of animals. Moreover, in the same animals, buccal hyperkeratotic lesions were seen adjacent to Co_5Sm magnets and the magnets could not be excluded as the cause of the lesions. It was concluded, however, that corrosion products from the magnets rather than the magnetic fields was the causative agent (Linder-Aronson *et al.*, 1995b; Camillieri and McDonald, 1993; Bondemark *et al.*, 1994), although *in vitro* studies have indicated that a static magnetic field may have a direct inhibitory effect on cell growth (Linder-Aronson and Lindskog, 1995). This, as well as effects from corrosion products, however, require close contact between magnets and cells or tissues. The clinical situation presents itself somewhat differently in that the magnets are often situated some distance away from the gingiva and bone. It is well known that the strength of a magnetic field drops exponentially with distance (Cerny, 1978; Linder-Aronson *et al.*, 1992; Bondemark and Kurol, 1992; von Fraunhofer *et al.*, 1992) and, consequently the previously observed biological effects may not be observable in an experimental situation mimicking the clinical environment. Thus, the present experimental study was undertaken to test this hypothesis using commercially available Co_5Sm magnets for orthodontic treatment in comparison with Neo Sentalloy closed coil springs with respect to possible side effects on alveolar bone growth, gingival epithelial thickness as well as rate of space closure.

Materials and methods

Experimental procedure

Four male monkeys (*Macaca fascicularis*) were used in the experiment. On the basis of their dental development (Hurme and Van Wagenen, 1961) two of the monkeys (I and II) were estimated to be 3–4 years old and the other two (monkeys III and IV) 6–7 years. During the experimental period the monkeys were fed standard pelleted food (Astra Ewos, Södertälje, Sweden) supplemented with fruit. The monkeys were anaesthetized with 0.5 ml (10 mg/kg b/w) ketamine HCl (Kethalar®; Park Davies, Morris Plains, NJ, USA) prior to each experimental procedure and check-up.

Intraoral photographs and impressions in alginate were taken 2 weeks prior to any experimental device being introduced. Furthermore, in monkeys I and II the second premolars and in monkeys III and IV the first molars were extracted at this time to give room for the orthodontic appliances. Full crowns with brackets fitted on the buccal surfaces were manufactured in gold (JS C-gold®; Sjödings Dentalprodukter AB, Solna, Sweden) for all first molars and first premolars in monkeys I and II and for all second molars and second premolars in monkeys III and IV. Buccal extensions towards the sulcus were made in the second and fourth quadrants, the premolars having the extensions mesial to the brackets and the molars distal to the brackets (Fig. 1). To increase retention, grooves were cut with a burr in the enamel of the teeth to be crowned in monkeys I and II. In monkeys III and IV additional fixation of the appliances was achieved by screwing threaded gold pins through the lingual wall of the gold crowns into holes drilled in the teeth. The teeth in all monkeys were etched with 30 per cent phosphoric acid for 30 seconds, rinsed with water and dried. The crowns were cemented with carboxylate cement (Durelon®; Espe, Seefeld, Germany) and 2 days later fitted with orthodontic appliances as described below.

Figure 1 The magnetic and coil spring appliances *in situ*.

The animals were injected i.m. with 150 mg/kg b/w doxycyclin (Vibramycin; Pfizer, Amboise, France) at the start of the experiment. Professional oral hygiene procedures and inspection of the appliances were performed once a week. The animals were killed after 13 weeks by an overdose of ketamine HCl.

Magnetic appliance

A passive 0.016″ round Australian wire (T.P Laboratories Inc., La Porte, Ind., USA) was ligated with steel ligatures to the brackets on the gold crowns on both sides of the extraction spaces in the second and fourth quadrants. The magnetic unit consisted of two Co_5Sm magnets ($4 \times 5 \times 2$ mm) mounted on a bar along which they could slide freely. Each magnet was encased in a stainless steel jacket with the exception of the uncovered attracting pole faces (2×5 mm) (Medical Magnetics Inc., NY, Ramsey, USA). The bar with the magnets was inserted and fixed in holes on the buccal extensions of the gold crowns. In order to create an attracting force to close the extraction spaces, the magnets were separated 3 mm and ligated to the extensions with 0.003″ steel ligatures (Dentaurum, Pforzheim, Germany) (Fig. 1). The ends of the ligatures were carefully hidden to avoid mechanical wounding of surrounding soft tissues.

Coil spring appliance

A passive 0.016″ × 0.016″ square stainless steel wire (Blue Elgiloy, Rocky Mountains Inc., Denver, CO, USA) was ligated with steel ligatures to the brackets on the gold crowns on both sides of the extraction spaces in the first and third quadrants. The ends of the ligatures were carefully hidden to avoid mechanical wounding of surrounding soft tissues. Sliding archwire hooks (Unitek, Monrovia, CA, USA) were mounted on the wire mesial to the premolar bracket and distal to the molar bracket. A light superelastic Sentalloy (Superelastic Nickel Titanium Alloy) closed coil spring (GAC International Inc., Central Islip, NY, USA) was attached to the hooks in each quadrant (Fig. 1). The coil spring exerted an attracting force on the teeth in order to close the extraction space.

Histological preparation

After sacrifice, tissue blocks containing jaw segments including all premolars and molars were dissected out and fixed in 5 per cent neutral-buffered formalin for 48 hours. After fixation, the jaw segments were dehydrated and embedded in a low-viscosity embedding medium (Spurr; Agar Aids Ltd., Stansted, England). The blocks were trimmed and 50 μm sections were cut with a diamond saw (Exact Apparatebau; Otto Herrmann, Norderstedt, Germany) bucco-lingually through the alveolar processes (Fig. 2). Every second ground section was stained with Methylene Blue-azure II and basic fuchsine according to Humphrey and Pittman (1974) to visualize the epithelium. The sections were mounted with synthetic mounting medium (Eukitt; O. Kindler, Freiburg, Germany) and examined independently by two investigators in a light microscope (Aristoplan; Ernst Leitz GmbH, Wetzlar, Germany) under incident ultraviolet and transmitted polarized light. Morphometrical recordings were made with a computerized image analysis package (Argus-50; Hamamatsu, Hamamatsu City, Japan).

Histological and histomorphometrical evaluation

Measurements of newly formed bone were made using the lines with tetracycline (TC) incorporation in five different predetermined locations (Fig. 3): the buccal bone under the marginal, the middle and the apical parts of the appliance; on the lingual surface of the alveolar process; and in the cancellous bone. The total bone width under the appliances was also measured in all buccal and lingual locations.

Descriptive and quantitative recordings were made of the number of parallel and single TC-lines, the presence of TC-lines buccally under the appliances, towards the tooth (or extraction space), around blood vessels and in the cancellous bone. The amount of buccal

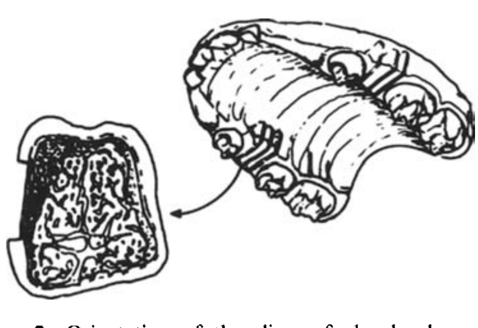

Figure 2 Orientation of the slices of alveolar bone cut through the alveolar processes.

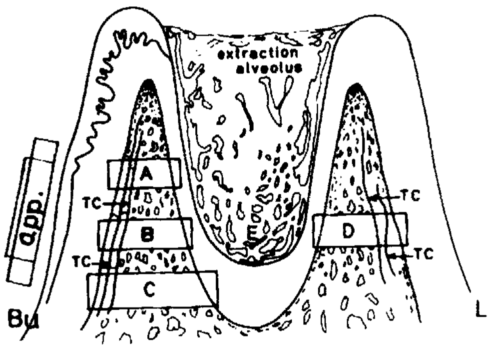

Figure 3 Bucco (Bu)-lingual (L) section and the position of the appliance (app). Measurements were made of newly formed bone between the two expected lines with tetracycline (TC) incorporation (arrows) in five different locations: the buccal bone under the marginal (**A**), the middle (**B**), and the apical (**C**) part of the appliance, on the lingual surface of the alveolar process (**D**) and in the cancellous bone (**E**).

periosteal resorption under the appliances (B in Fig. 3) was assessed semi-quantitatively as 0, 1, 2 or 3. Also, the number of labelled osteocyte lacunae under the appliances (B in Fig. 3) were counted. Three sections per extraction space were used for the measurements; two adjacent to the teeth on either side of the extraction space and one section in the centre of the extraction space. The recordings were made twice by the same investigator with a 2-week interval. Mean values and SD were calculated as well as intra-examiner correlation.

The epithelial thickness was determined by counting the number of epithelial cell layers buccally in the free gingival groove between the attached and free gingiva (Berkovitz *et al.*, 1992) as well as buccally in the attached gingiva under the magnet or coil spring (opposite to B in Fig. 3). Every second section from the ten central-most sections in the extraction gap were evaluated. Each location was evaluated in three sites and all measurements were repeated after 2 weeks. The measurements were consistently made where the epithelium was at its thinnest in each site. Mean values, SD and intra-examiner correlations were calculated.

Measurement of space closure

The casts taken at the start of the experiment and when the appliances were removed were used to measure the amount of space closure. Each jaw was put into a stereograph (Schwartz, 1933; Linder-Aronson, 1960). A midline was constructed in the upper jaw using the mid-point between the central incisors on the lingual gingiva and two posterior points where rugae met in the midline (Fig. 4). In the lower jaw the mid-point between the central incisors on the lingual gingiva were used as well as a point half way between the most lingual parts of the lingual surfaces of the canines. A line was drawn between these points. From each cast the mid-points of the first premolar and molar in monkey I and II, and the mid-points of the second premolar and molar in monkey III and IV were transferred to a sheet of paper using the stereograph. From each point marked from the teeth a perpendicular line was drawn to the midline. The distances between premolar and molar was measured using a digital sliding caliper (Jocal; C.E. Johansson, Eskilstuna, Sweden) to an accuracy of 0.1 mm. The differences in distance between initial model and final

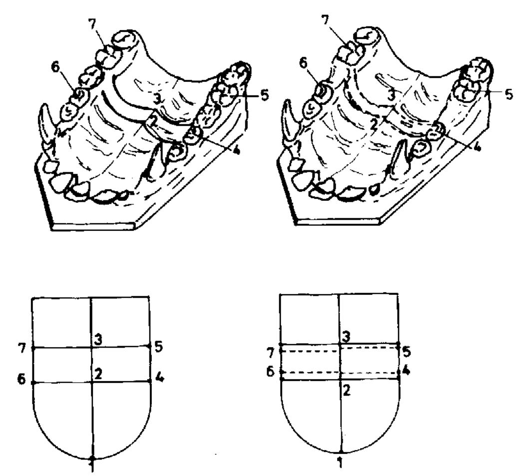

Figure 4 Casts from the upper jaw in monkey III used for stereographic measurements of space closure, at the start (to the left) of the experiment and when the appliances were removed (to the right). The following reference points were used for the measurements: (**1**) the point between the central incisors on the lingual gingiva, (**2** and **3**) the intersection between rugae and the midline, (**4**) the midpoint of the upper right second premolar (in monkey III and IV, first premolar in monkey I and II), (**5**) midpoint of the upper right second molar (in monkey III and IV, first molar in monkey I and II), (**6**) the midpoint of the upper left second premolar (in monkey III and IV, first premolar in monkey I and II), (**7**) the midpoint of the upper left second molar (in monkey III and IV, first molar in monkey I and II). The distance between the solid lines (before treatment) and the dotted lines (after treatment) represents space closure over the entire treatment period.

Table 1 Means with SD for clinical and selected histomorphometrical measurements. Significance levels (*P*) for differences between the two test groups, *n* denotes number of quadrants.

Variable	Unit	*n*	Magnet Mean	Magnet SD	Coil spring Mean	Coil spring SD	*P*
Rate of space closure	mm/month	8	0.78	0.25	0.97	0.32	>0.05
Periosteal resorption	arbitrary	8	2.17	0.86	1.17	0.79	<0.001
TC-labelled osteocyte lacunae	no. cells	8	66.3	28.6	27.7	12.8	<0.01
Epithelial thickness, free gingival groove	no. cells	8	20.7	2.1	20.2	1.6	>0.05
Epithelial thickness, attached gingiva	no. cells	8	21.6	1.8	21.2	1.9	>0.05

model represented space closure over the entire experimental period (Fig. 4). Furthermore, the rate of space closure was calculated. The material was measured on three different occasions by the same examiner. The two recordings exhibiting the best match were used for calculating a mean for each quadrant (Slagsvold, 1963). Mean values, SD (Table 1) and intra-examiner correlations (Table 2) were calculated.

Statistical analyses

Since the analysis of data was consistently based on matched pairs within the same jaw, Wilcoxon's signed ranks test for matched pairs (Siegel and Castellan, 1988) was used. Differences between the two groups were considered significant at $P<0.05$.

Results

Clinical evaluation

The monkeys tolerated the experiment well. On a few occasions recementation of lost crowns was performed. Recementation was done consistently within 1 day. In monkey I the gold crown on 24 had to be recemented after 6 weeks. On the same occasion, the ligature locking the magnet of 24 had to be replaced in monkey II. Moreover, the crown on 44 had to be recemented. In the same monkey the crowns on 44 and 46 were recemented after 8 weeks. In monkey III the appliance *regio* 25–27 had to be recemented after 2, 10 and 12 weeks. The appliance *regio* 35–37 was recemented after 3, 10 and 12 weeks and in *regio* 45–47 after 2 weeks. In monkey IV, the crown on 25 was recemented after 2, 6, 9 and 12 weeks.

Table 2 Intra-examiner correlation coefficients for repeated measurements.

Variable	Coefficient
Space closure	0.99
Tetracycline-labelled osteocyte lacunae	1.00
Epithelial thickness, free gingival groove	0.94
Epithelial thickness, attached gingiva	0.97

The magnets and the coil springs remained at a distance of 0.5–1 mm from the gingiva throughout the observation period. After 6 weeks corrosion of the uncovered areas of the magnets was evident (Fig. 5). All extraction spaces had been reduced at the time of sacrifice and no statistical difference between magnet and coil spring appliances could be found with respect to rate of space closure (Table 1). The intra-examiner correlation for repeated measurements of space closure was high (0.99, Table 2).

Histological and histomorphometrical evaluation

Intra-examiner correlations for repeated measurements of bone measurements and epithelial

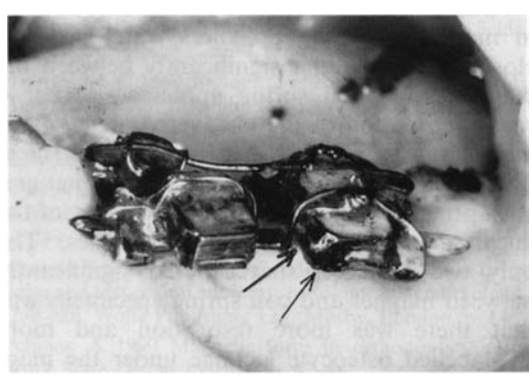

Figure 5 Corrosion of the uncovered pole faces of the magnets at the end of the experimental period (arrows).

thickness were high and in general consistently >0.90 (Table 2).

Bone measurements

No statistical differences were found between the magnet and coil spring specimens with respect to any of the linear measurements between TC-lines. The only two variables that differed significantly between magnet and coil spring specimens were periosteal bone resorption and number of TC-labelled osteocyte lacunae under the appliances (Table 1). Both were higher under the magnets.

Epithelial thickness

In all specimens, a healthy buccal gingival epithelium without any signs of wounding or inflammation was seen. No statistical differences could be found between magnet and coil spring specimens with respect to epithelial thickness (Table 1).

Discussion

The present experimental study was designed to mimic the clinical situation in orthodontic space closure with magnets or coil springs. The two methods were compared with respect to rate of space closure. Furthermore, it was the aim to detect any possible side effects of orthodontic rare earth magnets on alveolar bone growth and gingival epithelial thickness. Monkeys were chosen as experimental animals to enable application of the same type of appliances used clinically, as well as to allow quantitative histomorphometric evaluation of alveolar bone formation and epithelial thickness in the vicinity of the appliances.

Although some difficulties were encountered in maintaining the appliances, rate of space closure did not differ significantly between the magnetic and coil spring appliances. After 6 weeks, corrosion of the uncovered areas of the magnets was already evident. No statistical differences were found between the magnet and coil spring specimens with respect to any of the linear measurements between TC-lines. The only two variables that differed significantly between magnet and coil spring specimens was that there was more resorption and more TC-labelled osteocyte lacunae under the magnets. However, no statistical difference could be detected between magnet and coil spring specimens with respect to epithelial thickness. Although subtle in magnitude, the possible significance of these cell and tissue reactions will be addressed first, followed by any possible clinical implications thereof.

Previous studies with rare earth magnets in close contact with skin and bone have demonstrated statistically significant reductions in epithelial thickness and bone formation (Linder-Aronson and Lindskog, 1991; Linder-Aronson *et al.*, 1992, 1995a; Linder-Aronson and Rygh, 1994) as well as an increase in bone resorption (Linder-Aronson and Lindskog, 1991). In the present study only increased bone resorption close to the magnets could be confirmed. However, other studies have also failed to detect any significant effects on bone formation (Camilleri and McDonald, 1993). The subtle effects on bone tissue in the present study as well as others (Linder-Aronson and Lindskog, 1991; Linder-Aronson *et al.*, 1992, 1995a; Linder-Aronson and Rygh, 1994) may be explained by the design of the experimental appliances. The magnets in this study were situated at a distance of 0.5–1 mm from the epithelium, sliding on a steel bar with the magnetic field mainly parallel to the underlying tissues. Differences in the direction of the magnetic field has been proposed as factor influencing the biological effect (Gillings, 1993). However, this hypothesis was not confirmed by Papadopulos *et al.* (1992) or Camilleri and McDonald (1993). Furthermore, the magnetic field in the present study was time-varying as the magnets moved with respect to each other (Blechman, 1990) as opposed to magnetic fields in studies where significant effects on bone and epithelium have been recorded (Linder-Aronson and Lindskog, 1991; Linder-Aronson *et al.*, 1992, 1995a; Linder-Aronson and Rygh, 1994). Furthermore, both the epithelium, and more importantly, the bone surfaces were situated further away from the magnets and exposed to a considerably weaker magnetic field flux density than in previous studies (Linder-Aronson and Lindskog, 1991; Linder-Aronson *et al.*, 1992, 1995a; Linder-Aronson and Rygh, 1994). Thus, these experimental differences may explain the near lack of statistically significant effects of the magnets in the present clinical experimental situation and indicate negligible clinical side effects. Furthermore, based on the lack of difference in rate of space closure between magnet and coil spring appliances, the potentially cumbersome and expensive use of magnets in orthodontic treatment can be questioned provided the two types of appliances exert comparable forces.

An initial 3 mm distance between the two magnets was used in order to generate a force of ~ 1 N comparable to that of a light superelastic Sentalloy closed coil spring (Miura *et al.*, 1988; Gross, 1991; Han and Quick, 1992). However, a notable difference between coil springs and magnetic appliances is that the attracting force with magnets increase as the extraction space closes, while the force of a coil spring is more or less constant (Miura *et al.*, 1988; Gross, 1991, Han and Quick, 1992). Another important factor when comparing the present experimental situation to a clinical situation is that the forces exerted on teeth in the monkeys can not be directly transferred to forces used in humans with the same type of appliances. Human teeth are bigger than corresponding teeth in monkeys and the force per unit root surface area is higher in monkeys than in humans due to shorter roots and, consequently less root surface area in monkey teeth. Nevertheless, in clinical situations where eruption of impacted teeth are to be facilitated (Sandler *et al.*, 1989; Dean, 1990; Sandler and Springate, 1991; Vardimon *et al.*, 1991) and in some functional appliances (Vardimon *et al.*, 1989), use of magnets may present considerable advantages. However, corrosion (Tsutsui *et al.*, 1979; Vardimon and Mueller, 1985; Drago, 1991; Linder-Aronson *et al.*, 1995b) should be minimized by encasing the magnets more carefully. Furthermore, any potential effect of the magnetic field still needs to be considered. According to Gillings (1993) the effect of the field can be minimized by eliminating external magnetic fields using magnetizable alloy plates to abut the magnet pole faces in order to confine the direction of the external magnetic field and thus, provide a 'closed' field. This principle has been used in retention of overdentures (Gillings, 1993). Thus, it is recommended to use a plate of magnetizable alloy to attract one magnet instead of two attracting magnets for space closure to minimize possible adverse effects from the magnetic field.

In conclusion, although some marginal statistically significant differences were found between the magnet and coil spring specimens with respect to cell and tissue reactions, the present results indicate negligible clinical side effects of orthodontic magnets. From a clinical point of view, magnet and coil spring appliances were comparable with respect to rate of space closure.

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