European Journal of Orthodontics 20 (1998) 211–218

Long-term effects of orthodontic magnets on human buccal mucosa—a clinical, histological and immunohistochemical study

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SUMMARY The aim of this intra-individual study was to examine human buccal mucosa clinically, histologically and immunohistochemically after 9 months' exposure to orthodontic magnets.

In each of eight subjects (17.8–42.4 years), a magnet and a demagnetized equivalent of parylene coated neodymium-iron-boron was bonded alternately on the left and right side, to the buccal surface of maxillary premolars. The buccal mucosa was clinically examined and photographed, and three punch biopsies (6 mm diameter) of the buccal mucosa were taken from each subject at the site of contact with the magnet (test), in contact with the demagnetized magnet (control), and also at a site on the control side without contact with the demagnetized magnet (normal). The biopsies were snap-frozen for histological and immunohistochemical investigation, using antibodies to PD7 (lymphocyte 'naive T'), UCHL1 ('memory T'), HLADR, ELAM-1 and ICAM-1.

Clinically, the buccal mucosa showed normal features before and after the experimental period. In four of the eight subjects, the epithelium was 1.4–2.3 times thicker in the control and test tissues than in normal tissues. The difference between controls and tests was negligible, and there were no signs of increased keratinization or other surface abnormalities. Compared with normal sites, all control and test sites showed slightly increased ELAM-1/ICAM-1 vascular staining, accompanied in three subjects by small infiltrates of PD7/UCHL1-positive lymphocytic cells and in all subjects by focal keratinocyte HLADR-staining.

The minor tissue reactions found in test and control tissues were interpreted to be a result of microtrauma (contact irritation) and not caused by the static magnetic field *per se*, since there was no difference between test and control tissues.

Introduction

During the last decade the use of magnets for generating orthodontic forces has increased (Blechman, 1985; Dellinger, 1986; Sandler *et al*., 1989; Vardimon *et al*., 1991; Bondemark and Kurol, 1992; Darendeliler and Joho, 1993). When orthodontic magnets are used for tooth movements the oral tissues can be exposed to a static magnetic field with flux density values up to 170 millitesla (mT). However, the extent of the field is limited since the flux density decreases rapidly (exponentially) with increased distance from the magnets (Bondemark *et al*., 1995). Reports of possible biological effects of static magnetic fields have attracted interest but this topic is controversial due to the divergent reactions shown by cells and tissues exposed to the field. It has, in hypothetical models, been suggested that certain static magnetic fields may stimulate enzyme systems, cell turnover and osteogenesis *in vitro* and *in vivo* (Repacholi, 1985; United Nations Environment Programme/World Health Organization/International Radiation Protection Association, 1987; International Non-Ionizing Radiation Committee of the International

Radiation Protection Association, 1991; Camilleri and McDonald, 1993). Most of the reports on dental and medical magnets deal with screening cell culture *in vitro* tests and short-term *in vivo* tests in animals, and in general the short-term results indicate negligible or reversible aberrant biological effects with exposure of flux density values below 2 Tesla (United Nations Environment Programme/World Health Organization/ International Radiation Protection Association, 1987; International Non-Ionizing Radiation Committee of the International Radiation Protection Association, 1991).

Very little attention has been focused on the possible long-term effects of orthodontic magnets on oral tissues and since orthodontic treatment with magnets may continue for several months, it would be of particular interest to investigate possible biological effects of long-term exposure to orthodontic magnets on oral tissues.

Therefore, the objective of this study was to examine if 9 months' exposure to an orthodontic magnet could possibly induce any changes which would be clinically, histomorphologically and immunohistochemically detected in human buccal mucosa.

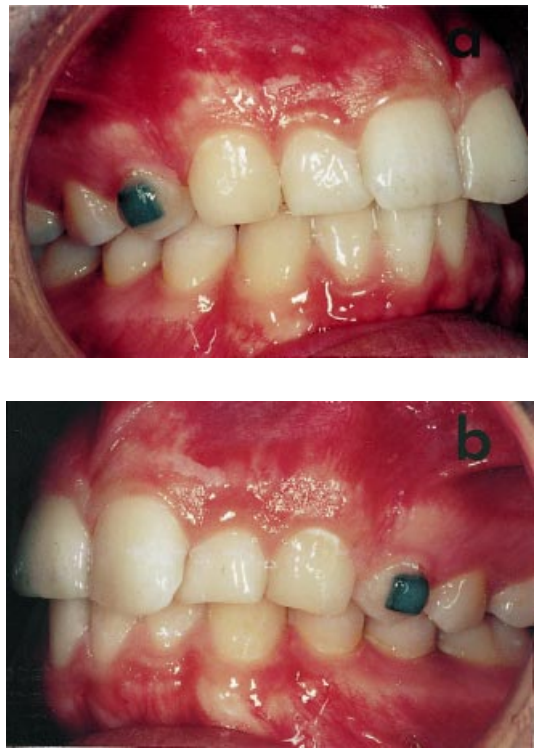

Figure 1 After 9 months' exposure. The control side (a), with a demagnetized magnet sealed and bonded with composite buccally on a maxillary first premolar, and the test side (b) with a magnet sealed and bonded with composite on the contralateral premolar.

Subjects and methods

Eight healthy subjects, six women and two men (mean age 29.1, SD 11.31, range 17.8–42.4 years), participated in the experiment. In each subject, a magnet was bonded with a thin layer of composite (Concise 3M, Medica GmbH, Borken, Germany) to a premolar on one side and a demagnetized magnet on the contralateral tooth with the pole face in contact with the buccal mucosa. The magnet bodies consisted of parylene coated neodymium-iron-boron ($Nd_2Fe_{14}B$, $3 \times 3 \times 1$ mm, Magnet Developments Ltd, Swindon, UK). On average, the bucco-lingual thickness of the bonded magnets and demagnetized magnets, including the coating layer of composite amounted to 2 mm (Figure 1).

For measurements of the flux density values (magnetic field) of the magnets, a gaussmeter with a Hall probe (Magnetic Instrumentation Inc., 7300 312R, Indianapolis, Indiana, USA) was used. In all subjects the field exposure of the test biopsies ranged from 80 to 140 mT. The distance between the magnet and the contralateral control and normal biopsy sites exceeded 40 mm in all subjects. As a result, the contralateral control and normal biopsy sites were exposed to flux density values of < 0.05 mT, i.e. an exposure equivalent to the level of the natural magnetic field of the earth.

Before bonding and after the experimental period, the buccal mucosa was clinically examined and photographed. At the end of the 9 months' experimental period, three punch biopsies were taken under local anaesthesia (Xylocaine adrenaline, Astra, Södertälje, Sweden) from each subject at the site of contact with the magnet (test), the site of contact with the demagnetized magnet

(control), and at a site on the control side without contact with the demagnetized magnet (normal). A 4 per cent erythrosine solution was painted onto the surface of the magnet bodies for identification of the mucosa in contact with the magnet bodies and a 6-mm punch biopsy (Stiefel Laboratories Ltd, Wooburn Green Bucks, UK) was then taken. The normal biopsy of the subject was taken of unstained buccal mucosa on the control side and at least 10 mm away from the stained area.

Biopsy preparation and staining

The punch biopsies were embedded in OCT (Miles Inc.) and snap-frozen in isopentane at –80°C. Frozen serial sections (6 μm) were air-dried, fixed for 10 minutes in acetone and washed in phosphate buffered saline (PBS) followed by 0.3 per cent H_2O_2 in PBS for 30 minutes to block endogenous peroxidase. The sections were then incubated for 60 minutes at room temperature with the following antibodies at optimal dilutions: monoclonal anti-human leukocyte antigen DR (HLADR, clone CR3/43, DAKO A/S, Copenhagen, Denmark), anti-PD7 ('naive T', CD45RB, DAKO), anti-UCHL1 ('memory T', CD45R0, DAKO), anti-intercellular adhesion molecule-1 (ICAM-1, CD54, Immunotech), and anti-endothelial leukocyte adhesion molecule-1 (ELAM-1, CD62E, Immunotech). After a brief rinse in PBS, the sections were incubated with peroxidase-conjugated rabbit-anti-mouse IgG (1:50) and peroxidase activity was detected with 0.05 per cent DAB supplemented with 0.01 per cent H_2O_2 (30 minutes). Sections were counterstained with haematoxylin and mounted. Control sections were incubated without the primary antibodies. The first and the last sections of the series were stained with haematoxylin and eosin for routine histomorphological analysis.

Using a graded eye-piece, the epithelial thickness in test and control mucosa was measured relative to the normal mucosa of each subject. The epithelial thickness was defined as the distance from the epithelial surface to the deepest level of the rete pegs. The thickness was calculated in each individual biopsy as the mean of two measuring points.

Ethical considerations

Informed consent was obtained from all subjects after presenting written information, and the study was approved by the Ethical Commitee of the University of Lund, Sweden.

Results

Clinical assessments

The buccal mucosa opposite all the magnet bodies showed normal features before and after the experimental period (Figure 2). In one subject an area, approximately 2 × 2 mm was found where the magnetic alloy, from the control, was exposed to intra-oral corrosion assaults.

Histological evaluation

Routinely-stained sections. No changes could be demonstrated in any of the biopsies regarding aberrant keratinization, erosions, ulcerations or other surface abnormalities and no heavy infiltration of inflammatory cells could be detected except in one control biopsy. In four subjects the epithelium was 1.4–2.3 times thicker in the test and control biopsies, than in comparison with the normal biopsies (Figure 3). The difference in epithelial thickness between test and control biopsies was negligible.

Immunostained sections

General observations—Well preserved sections for immunohistochemical analysis of the different antibody stain reactions were retrieved from all 24 biopsies.

PD7 ('naive T') and UCHL1 ('memory T')—In three subjects the test and control biopsies showed slightly more PD7/UCHL1-positive cells than the corresponding normal biopsies. The cells were located in the connective tissue papillae, at small vessels or as occasional small foci adjacent to the epithelium (Figure 4). The difference between test and control biopsies was negligible. However, in one subject the control biopsy, facing the magnet body with exposed neodymium-iron-boron alloy, showed extensive infiltrates of

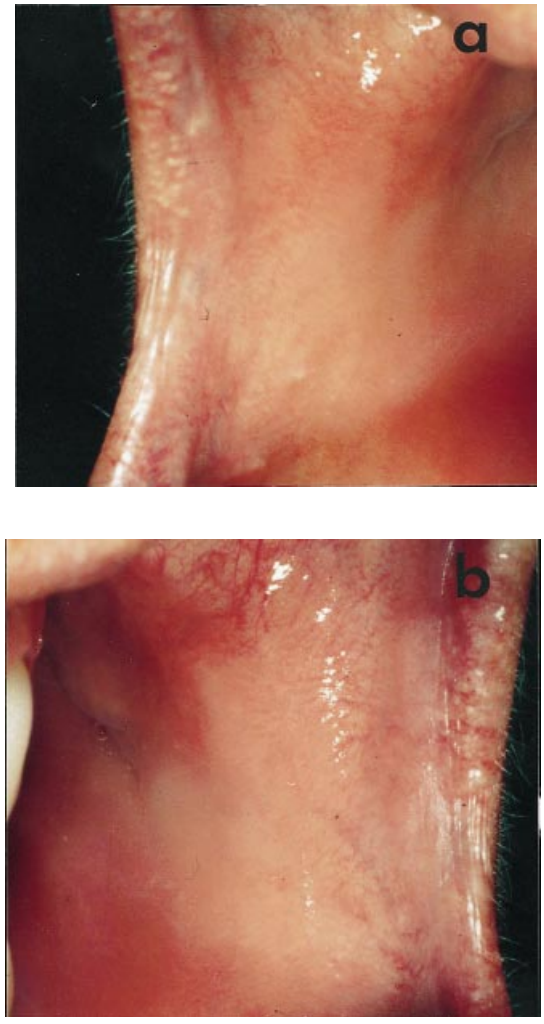

Figure 2 After 9 months' exposure. (a) The control mucosa opposite the demagnetized magnet and (b) the corresponding test mucosa opposite the magnet of the same subject. Both test and control mucosa showed normal features.

PD7/UCHL1-positive cells. In the remaining four subjects the test and control biopsies differed little from the PD7/UCHL1-findings in normal mucosa.

HLADR—In the test and control tissues the HLADR-stained Langerhans cells (LC) tended to arrange themselves towards the tips of the connective tissue papillae, and the connective tissue dendritic cells to align themselves along

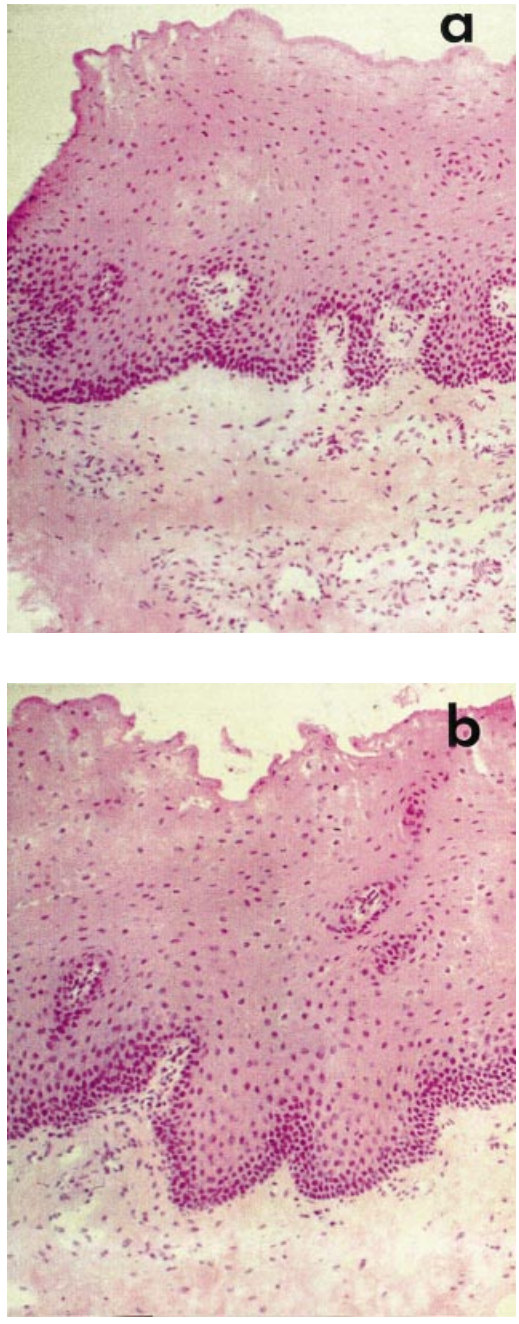

Figure 3 Frozen section of (a) normal mucosa, without contact with the demagnetized magnet, and (b) the corresponding test site of the same subject, showing an increased epithelial thickness at the test site, exposed to the magnet. No surface aberrations could be detected. The control site of this subject, exposed to a demagnetized magnet, showed a similar increased epithelial thickness, lacking surface changes. Haematoxylin-eosin, magnification ×100.

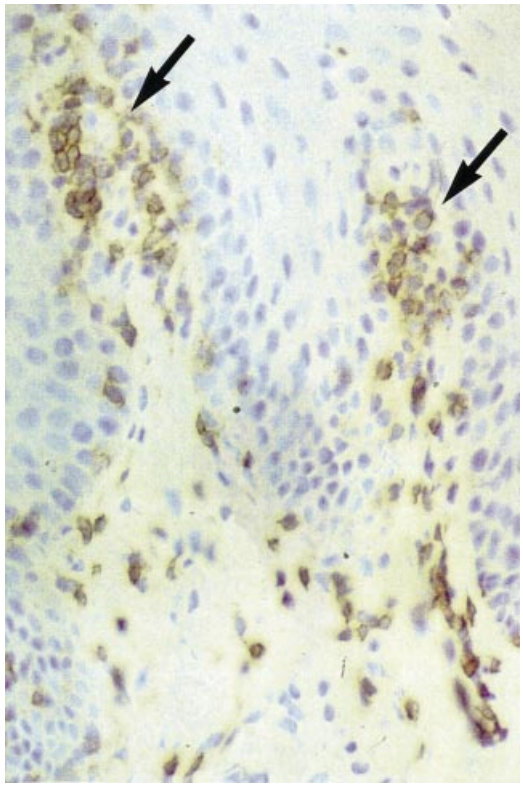

Figure 4 Frozen section of control site (demagnetized magnet) incubated with PD7-antibody ('naive' T cells). Small infiltrates of stained cells are seen in connective tissue papillae (arrows), with only an isolated cell in the epithelium. Similar PD7-stained infiltrates were seen at the test site (magnet) of this subject, but not in the normal mucosa. These infiltrates also showed UCHL1-stained cells ('memory' T). Magnification ×330.

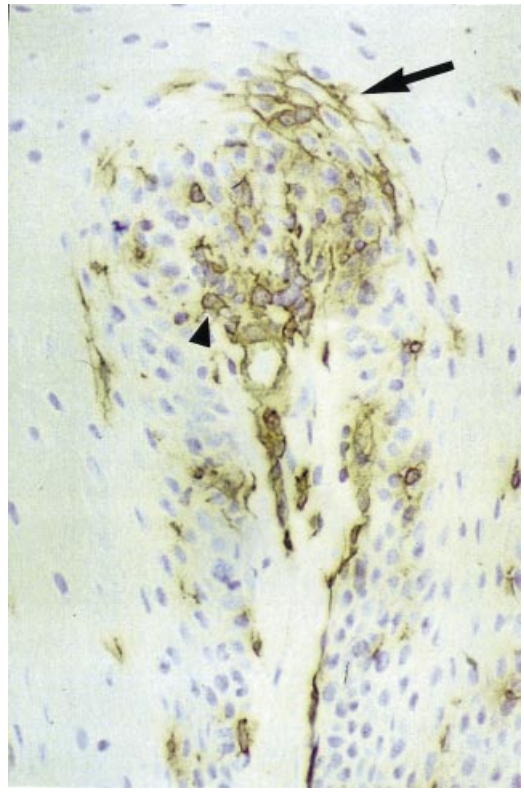

Figure 5 Frozen section of test site (magnet) incubated with HLADR-antibody. In the connective tissue papillae, staining is seen in dendritic cells close to the epithelium, in vessel walls and in a few lymphocyte-like cells (arrow head). In the adjacent epithelium, a few dendritic, LC-like cells show staining as well as a focal area of keratinocytes (arrow), a reaction also seen at the control site (demagnetized magnet), but only occasionally at any of the normal sites. Magnification ×330.

the epithelial basal lamina. In addition, focal areas of HLADR-stained keratinocytes (KC) were readily observed and exclusively at the tips of connective tissue papillae (Figure 5). Such findings were only occasionally found in normal tissue. There was no differences in the HLADR-staining reactions between test and control tissues. An exception to this was the control biopsy in the subject with extensive infiltration of PD7/UCHL1. In this control biopsy, an extensive HLADR epithelial staining was observed.

ELAM-1—In comparison with normal biopsies the test and control biopsies showed a pattern of slight ELAM-1-stained small vessels in the connective tissue papillae (Figure 6). No difference in staining was found between tests and controls except in one subject, where the control biopsy (the same as showed extensive infiltrations of PD7/UCHL1-positive cells and extensive HLADR staining) showed exceptionally prominant vascular staining associated with heavy infiltrates of mononuclear cells.

ICAM-1—The test and control tissues showed slightly accentuated vascular staining, particularly in the connective tissue papillae, where vessels stained more prominantly than in any of the

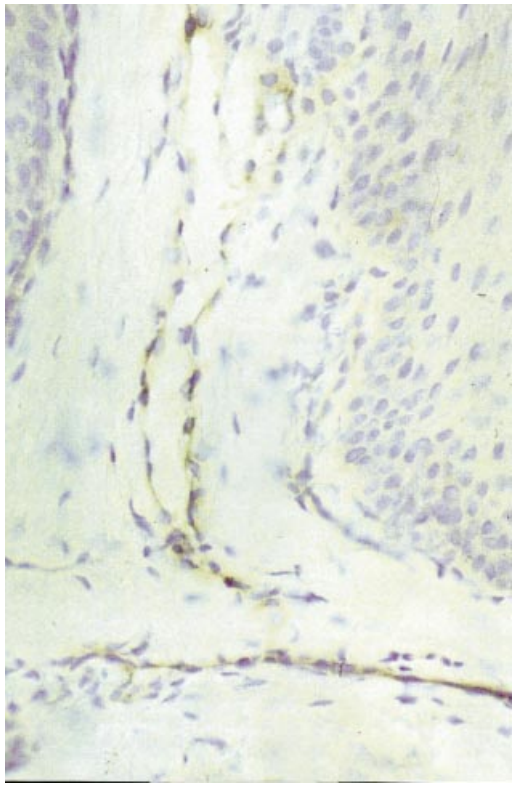

Figure 6 Frozen section of test site (magnet) incubated with ELAM-1-antibody. Staining is seen in the endothelium in small thin-walled vessels projecting into the connective tissue papillae. Similar ELAM-1-staining was also seen at the control site (demagnetized magnet), but not in normal mucosa. Magnification ×330.

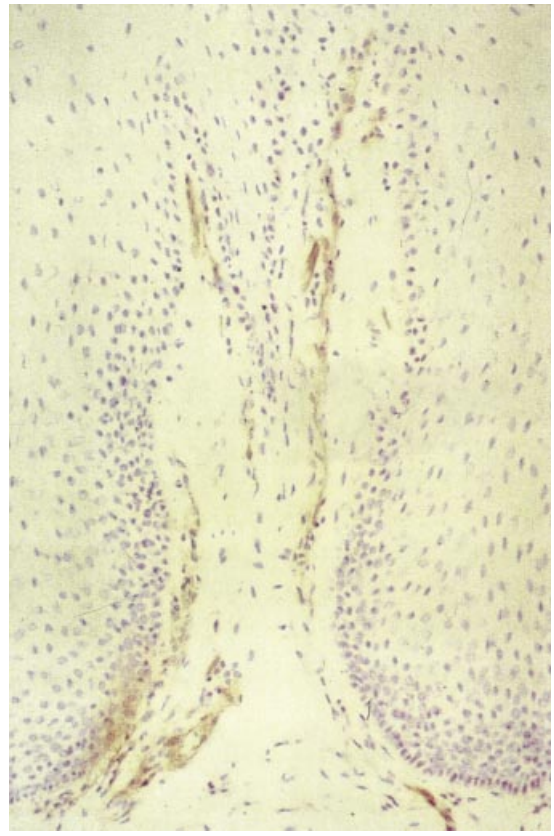

Figure 7 Frozen section of test site (magnet) incubated with ICAM-1-antibody. Vascular endothelial staining is present in small vessels projecting into the connective tissue papillae. Note the absence of staining in adjacent epithelium. Similar vascular staining was seen at the control site (demagnetized magnet) but was less prominent in normal mucosa. Magnification ×160.

normal sites (Figure 7). The difference in staining between tests and controls was negligible. However, in one subject, the one with extensive HLADR and ELAM-1-staining and extensive infiltration of PD7/UCHL1-positive cells, the control biopsy also showed strong vascular ICAM-1-staining.

Discussion

The findings show that minor tissue reactions may occur in the form of increased thickness of the epithelium of the oral buccal mucosa, after 9 months' contact with a small magnetic body generating a static magnetic field, as well as after contact with a small demagnetized magnetic body. Our interpretation is that the increased epithelial thickness may be due to frictional microtrauma (contact irritation) caused by the extension of the magnet body from the smooth buccal surface of the tooth. Frictional trauma to oral epithelium is known to occasionally result in an increased rate of cell proliferation and epithelial thickening (MacKenzie, 1980).

The purpose of the immunohistochemical analysis was to detect any additional, subtle changes which could not be observed in routinely-stained

sections. The choice of antibodies was made to establish if any changes were secondary to, or a consequence of, 'irritation' of the epithelium due to its close contact with the magnet body. The increased epithelial thickness was found to be accompanied by a minor lymphocytic infiltration and vascular expression of ICAM-1 and ELAM-1, as well as by a slight degree of HLADR-expression by adjacent keratinocytes and Langerhans cells in the test and control biopsies. In normal tissue, this was only rarely observed. Keratinocytes are a potential source of cytokines (Nickoloff and Turka, 1994), some of which may induce changes not only at the epithelial but also at the connective tissue level. It is possible that the irritated epithelium may increase its production of cytokines such as interleukin-1 (IL1), and this may contribute to an increased expression of ICAM-1 locally in the vessels (Kupper and Groves, 1995). Furthermore, it has been reported that ELAM-1-expression in oral mucosa is associated with inflammatory conditions (Nylander *et al*., 1993; Tonetti *et al*., 1994; Gemmel *et al*., 1994), and it has been shown that there is a tendency to prolonged *in vivo* expression associated with chronic inflammatory lesions (Keelan and Haskard, 1992). Moreover, ELAM-1 seems to efficiently mediate adhesion of memory type T-cells (Picker *et al*., 1991; Shimizu *et al*., 1991). These background data suggest that our findings of a slightly induced expression of ELAM-1 and ICAM-1 in test and control biopsies could be due to frictional irritation of the mucosa by the magnet body.

A separate finding in this study was that a single subject showed extensive infiltration at the control, but not at the test site. This is interpreted as being the result of microtraumatic ulceration caused by friction, and exposure to potentially cytotoxic corrosion products. This was the only case where the magnet coating was breached, exposing the magnet alloy to saliva. Rare earth magnet alloys, such as neodymium-iron-boron, as used in this study, are known to be easily corroded by saliva (Vardimon and Mueller, 1985). When the alloy corrodes, there is a considerable risk of liberation of potentially cytotoxic components (Sandler *et al*., 1989; Bondemark *et al*., 1994; Donohue *et al*., 1995). Therefore, to avoid intra-oral corrosion, magnets have to be coated with, for example, composite.

The ultimate biological test is the human clinical evaluation. However, in contrast to animal studies, ethical and technical reasons often make it difficult to standardize the experiment. Therefore, it is not meaningful to submit immunosections to a quantitative analysis such as counting cells or cell layers, and furthermore, statistical calculation of such results would not be appropriate. Instead, in this study, a common histological approach with an intra-individual experimental protocol was used where the cellular and staining pattern of a test biopsy (exposed to a magnet) and of a control biopsy (exposed to a demagnetized magnet) were intra-individually compared and related to the appearance of a normal tissue biopsy, i.e. a reference biopsy from an area which had not been exposed to magnet body or a magnetic field.

In summary, in this intra-individual human study minor tissue reactions could be demonstrated in the buccal mucosa for both test and control sites following exposure (9 months) to magnetized and demagnetized magnets. These were interpreted as the result of microtrauma (contact irritation) due to the extension of the magnet body, and not to the effects of the static magnetic field *per se*, since there was no difference between test and control tissues.

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Acknowledgement

The research was supported by a grant from Swedish Dental Society.

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