

An M-CSF Receptor c-Fms Antibody Inhibits Mechanical Stress–Induced Root Resorption during Orthodontic Tooth Movement in Mice

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ABSTRACT

Objective: To examine the effect of anti-c-Fms antibody on odontoclastogenesis and root resorption in an orthodontic tooth movement mouse model.

Materials and Methods: We used orthodontic tooth movement in which an Ni-Ti coil spring was inserted between the upper incisors and the upper first molar. Root resorption occurred in this model. Anti-c-Fms antibody was injected daily into a local site for 12 days during mechanical loading. Odontoclastogenesis and root resorption were assessed by histology and scanning electron microscopy.

Results: The anti-c-Fms antibody significantly inhibited odontoclastogenesis and root resorption during orthodontic tooth movement.

Conclusion: M-CSF and/or its receptor is a potential therapeutic target in mechanical stress–induced odontoclastogenesis, and injection of an anti-c-Fms antibody might be useful for inhibition of mechanical stress–induced root resorption during orthodontic tooth movement. (*Angle Orthod.* 2009;79:835–841.)

KEY WORDS: c-Fms; Odontoclast; Root resorption; Mouse; Orthodontics

INTRODUCTION

The formation of osteoclasts depends on macrophage colony-stimulating factor (M-CSF) and a ligand for the receptor activator of necrosis factor κ B (RANKL). It has been reported recently that adminis-

tration of an antibody against the M-CSF receptor c-Fms (anti-c-Fms antibody) blocked osteoclastogenesis and orthodontic tooth movement.^{1,2}

Root resorption often is observed as an undesirable side effect of orthodontic treatment. It is a very serious problem for most clinicians, but it is still not understood. The cause of root resorption is considered to be multifactorial. Several studies have suggested that excessive orthodontic force is a critical factor for the root resorption.^{3,4} Associations between root resorption and tooth morphology,⁵ tooth intrusion,^{6,7} periodontal condition,⁸ or systemic factors such as genetics,⁹ the immune system,^{10,11} and bone metabolism.^{12,13} have been suggested. The cells responsible for the resorption of tooth roots are called *odontoclasts*. These cells are considered to be similar to osteoclasts.

Osteoclasts, which differentiate from hematopoietic stem cells, are required for bone resorption and remodeling. Formation of osteoclasts is dependent on M-CSF and a ligand for RANKL. Both M-CSF and RANKL are essential factors for osteoclast differentiation.¹⁴ Tumor necrosis factor- α (TNF- α) also was reportedly able to induce osteoclasts from bone marrow macrophages in vitro.^{15–17} TNF- α –induced osteoclast recruitment is probably central to bone erosive diseases such as rheumatoid arthritis,¹⁸ periodontal disease,¹⁹ periprosthetic bone loss,²⁰ and postmenopausal oste-

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opporosis.²¹ Several studies have shown that excessive orthodontic force induces expression of TNF- α and suggests an important role of TNF- α in orthodontic tooth movement.^{22–24} We also investigated the role of TNF- α in orthodontic tooth movement by using TNFR-deficient mice. Results showed that tooth movement in TNFR-deficient mice was less than that in wild-type mice, and that TNF- α plays an important role in mechanical loading-induced osteoclastogenesis and bone remodeling in orthodontic tooth movement.²⁵

It has been reported recently that administration of an antibody against the M-CSF receptor c-Fms (anti-c-Fms antibody) completely blocked osteoclastogenesis and bone erosion induced by TNF- α administration or inflammatory arthritis.¹ Because anti-c-Fms antibody inhibited TNF- α -induced osteoclast recruitment, we investigated whether the anti-c-Fms antibody could inhibit mechanical stress-induced osteoclastogenesis and orthodontic tooth movement in mice. The anti-c-Fms antibody significantly inhibited orthodontic tooth movement, markedly reducing the number of osteoclasts *in vivo*.² These findings suggest that M-CSF plays an important role in mechanical loading-induced osteoclastogenesis and bone resorption during orthodontic tooth movement mediated by TNF- α . Because root resorption is understood to be performed by odontoclasts, similarly to osteoclasts in bone resorption, we hypothesized that the anti-c-Fms antibody might inhibit odontoclastogenesis and root resorption during orthodontic tooth movement.

In the present study, we demonstrate the effect of anti-c-Fms antibody on mechanical stress-induced odontoclast and the degree of bone resorption assessed using an orthodontic tooth movement mouse model.

MATERIALS AND METHODS

Experimental Animals

Wild-type mice (male C57BL6/J) were purchased from Japan SLC Inc (Shizuoka, Japan). Eight-week-old mice were used in this experiment. The weights of mice ranged from 22 to 27 g. Animals were fed a granular diet (Oriental Yeast Co Ltd., Tokyo, Japan) to prevent them from exerting excessive chewing force. During the experiment, the mice were kept in cages in a room maintained at 25°C with a 12- to 24-hour light/dark cycle.

Experimental Tooth Movement

The mice were anesthetized with an intraperitoneal injection of 50 mg/kg pentobarbital sodium during setting and adjusting of the orthodontic appliance. The orthodontic appliance is composed of an Ni-Ti closed

coil spring. The appliance was inserted between the upper incisors and the upper left first molar and was fixed with a 0.1-mm stainless wire around both teeth.²⁵ According to the manufacturer's database, the force level of the coil spring after activation is approximately 10 \times g.²⁵ The left maxillary molar in each mouse was used to assess experimental tooth movement (Figure 1A). We evaluated odontoclast area by percentage of odontoclast surface on the pressure side of the distobuccal root of the first molar during orthodontic tooth movement (Figure 1B).

Preparation for Histologic Observation

After fixation, the maxillae were immersed in 10% EDTA for 10 days at 4°C for demineralization. Samples were embedded with paraffin and were sectioned at 4 μ m. Horizontal sections of the first molar region were prepared. Sections were obtained at a depth of 300 μ m of the first molar bifurcation using a microtome, and tartrate-resistant acid phosphatase (TRAP) staining was performed. For TRAP staining, sections were incubated in acetate buffer (pH 5.0) containing naphthol AS-MX phosphate (Sigma Chemical Co, St Louis, Mo), Fast Red Violet LB salt (Sigma), and 50 mM sodium tartrate. Sections were counterstained with hematoxylin. Odontoclast area was determined with the use of an NIH image.

Administration of Anti-c-Fms Antibody

AFS98, a rat monoclonal, anti-murine c-Fms antibody (IgG2a) that inhibits M-CSF-dependent colony formation and cell growth by blocking the binding of M-CSF to its receptor, has been described previously.²⁶ The AFS98 hybridoma was kindly supplied by Dr Shin-Ichi Nishikawa (Graduate School of Medicine, Kyoto University Medical School, Kyoto, Japan). The clone was maintained in HyQ-CCM1 medium (Hyclone), and the antibody was purified with the use of protein G (Sigma). Mice were injected daily for 12 days with 10 μ g anti-c-Fms antibody in 20 μ L PBS into a local site during orthodontic tooth movement.

Statistics

All data are expressed as means \pm SD, and statistical significance was calculated by one-way analysis of variance (ANOVA) and Scheffe's F-test.

RESULTS

Mechanical Loading Induces Odontoclastogenesis in an Orthodontic Tooth Movement Model

We assessed mechanical loading odontoclastogenesis during orthodontic tooth movement in a mouse

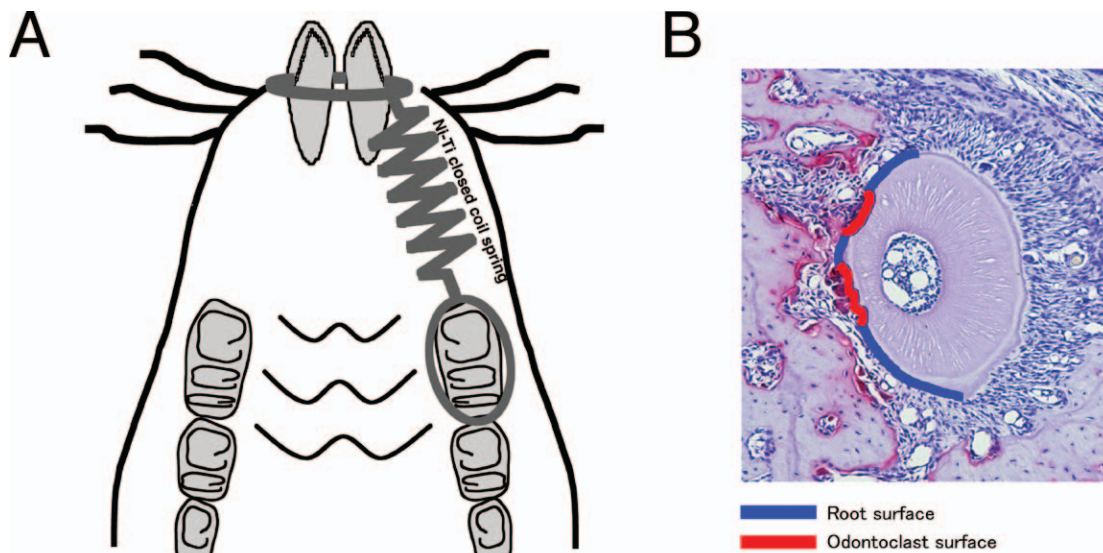


Figure 1. Schema of orthodontic tooth movement in mouse and evaluation of odontoclast area. The orthodontic appliance is composed of am Ni-Ti closed coil spring. (A) The appliance was inserted between the upper incisors and the upper left first molar and was fixed with a 0.1-mm stainless wire around both teeth. (B) Evaluation of the odontoclast area on the pressure side of the distobuccal root of the first molar during orthodontic tooth movement. Red line: half of the pressure side of the root surface. Blue line: odontoclast surface. Odontoclast area: percentage of Blue line/Blue line + Red line. Five sections, which were 100, 140, 180, 220, and 260 μm away from the bifurcation surface, per mouse, were measured for resorption area.

model. We observed histologic pictures of the distobuccal root of the first molar after it had been power loaded for 0, 2, 6, 10, or 12 days. From days 0 to 6, no odontoclasts were observed. On days 10 and 12, odontoclasts were observed on transverse histologic sections of the mesial side of the distobuccal root of the upper first molars (Figure 2).

Histologic Evaluation of the Inhibitory Effect of the Anti-c-Fms Antibody on Odontoclastogenesis during Orthodontic Tooth Movement

Figure 3 shows the histologic evaluation of odontoclastogenesis after application of mechanical force. We carried out TRAP staining on transverse histologic sections of the distobuccal root. The histology of TRAP staining of control mice, power-loaded mice, and power-loaded mice administered anti-c-Fms antibody on day 12 is shown in Figure 3. The odontoclast area was not observed on the root surface on the mesial side in control mice. Odontoclasts were found on the root surface in the mesial region in power-loaded mice on day 12. On the other hand, significantly fewer odontoclasts were found on the root surface of the mesial region in power-loaded mice administered anti-c-Fms antibody.

Electron Microscopic Evaluation of the Inhibitory Effect of the Anti-c-Fms Antibody for Root Resorption during Orthodontic Tooth Movement

Electron microscopic photographs of half of the mesial area of the distobuccal root of the first molar are

shown in Figure 4. Before tooth movement occurred, most of the roots exhibited areas covered by undamaged cementum with a characteristic smooth surface. By contrast, after tooth movement, root resorption was observed. However, in the anti-c-Fms antibody injection group, root resorption was prevented (Figure 4B).

DISCUSSION

Since the discovery of the RANK-RANKL signal transduction, RANKL has been thought to be essential for osteoclast differentiation. Recently, it was reported that $\text{TNF-}\alpha$ makes osteoclasts independent of RANKL.¹⁷ We investigated this using mice whose bone marrow stromal cells and osteoclast precursors are chimeric for the presence of TNF receptors, and found that both cell types mediated the $\text{TNF-}\alpha$ -induced osteoclastogenic properties.^{1,27} Furthermore, $\text{TNF-}\alpha$ induced M-CSF gene expression in stromal cells and increased osteoclast precursor numbers in vivo.¹ M-CSF assists in the survival and longevity of osteoclast precursors and organizes the cytoskeletons of osteoclasts.¹⁴ Recently, we reported that an anti-c-Fms antibody completely blocked pathologic osteoclastogenesis and bone resorption, whether in inflammatory arthritis or induced by direct injection of $\text{TNF-}\alpha$.¹ Therefore, M-CSF and c-Fms are clearly candidate therapeutic targets for $\text{TNF-}\alpha$ -related pathologic osteolysis.

It has been reported that orthodontic tooth movement increases the levels of $\text{TNF-}\alpha$ in the gingival sul-

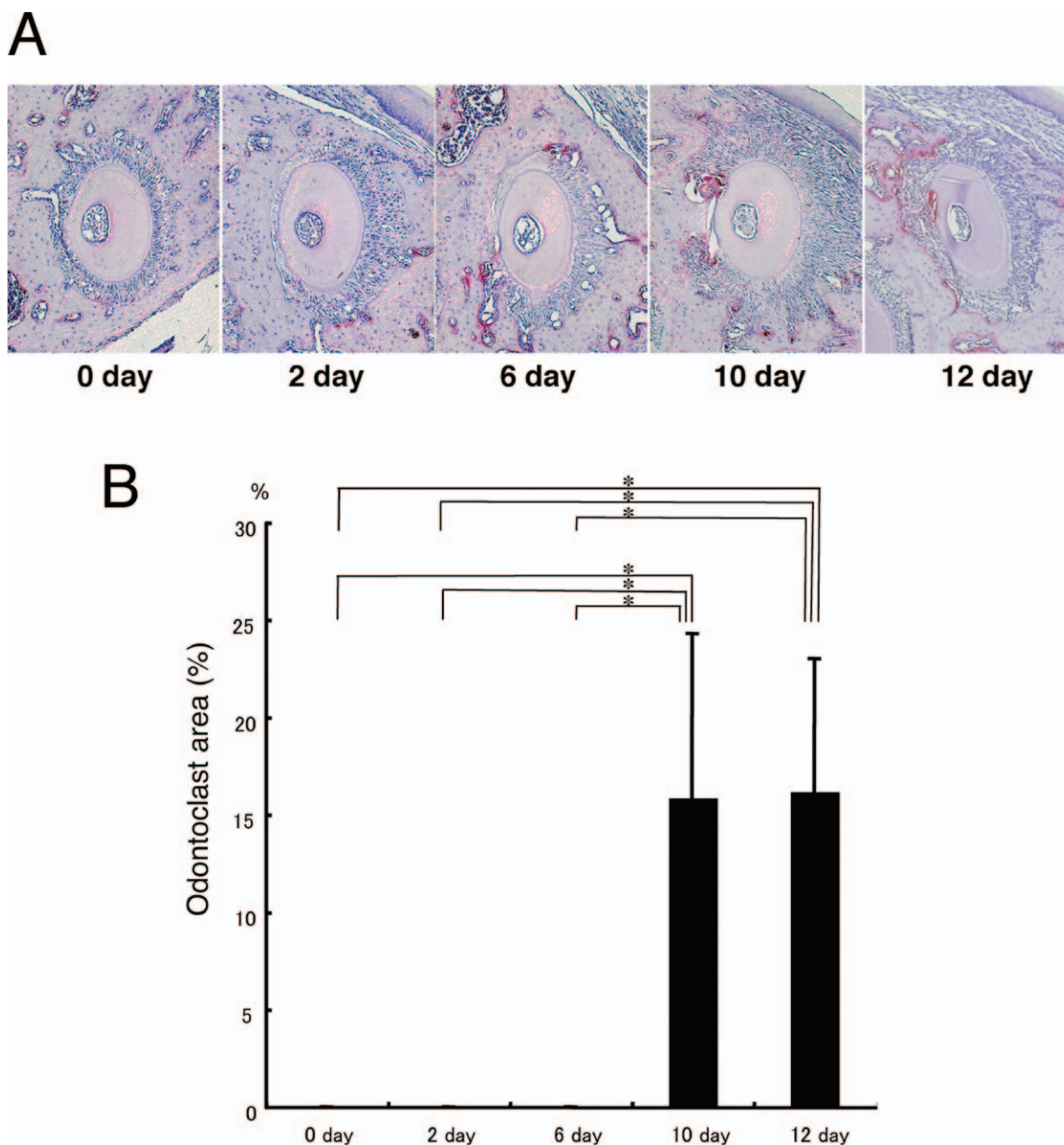


Figure 2. Time course of changes in the number of odontoclasts in an orthodontic tooth movement model. (A) Picture of changes in the number of odontoclasts in mice ($n = 5$ on all days). (B) Percentage of odontoclast area. $*P < .05$ by ANOVA and Scheffe's F-test. Results are expressed as means \pm SD.

cus in humans.^{23,24} Under pathologic conditions resulting from excessive orthodontic force, TNF- α has been shown to be expressed in rat periodontal tissue, suggesting an important role of TNF- α in orthodontic tooth movement.²² Recently, the anti-c-Fms antibody also inhibited TNF- α -related orthodontic tooth movement and markedly reduced the numbers of osteoclasts in vivo. Findings suggest that M-CSF plays an important role in mechanical loading-induced osteoclastogenesis and bone resorption during orthodontic tooth movement mediated by TNF- α . M-CSF basically is produced by mesenchymal cells, and its regulated secre-

tion has pathologic consequences for osteoclasts. For example, the absence of estrogen in postmenopausal osteoporosis is due to enhanced bone resorption caused by increased production of M-CSF by marrow stromal cells.²⁸ In inflammatory osteolysis, the level of M-CSF is increased in the serum of patients with rheumatoid arthritis who have severe ankylosing spondylitis²⁹ and in synovial fluid around loose joint prostheses.³⁰ These observations suggest that stromal cell-produced M-CSF may be an important mediator of osteoclastogenesis in vivo. In this study, we evaluated the effect of an anti-c-Fms antibody on mechanical

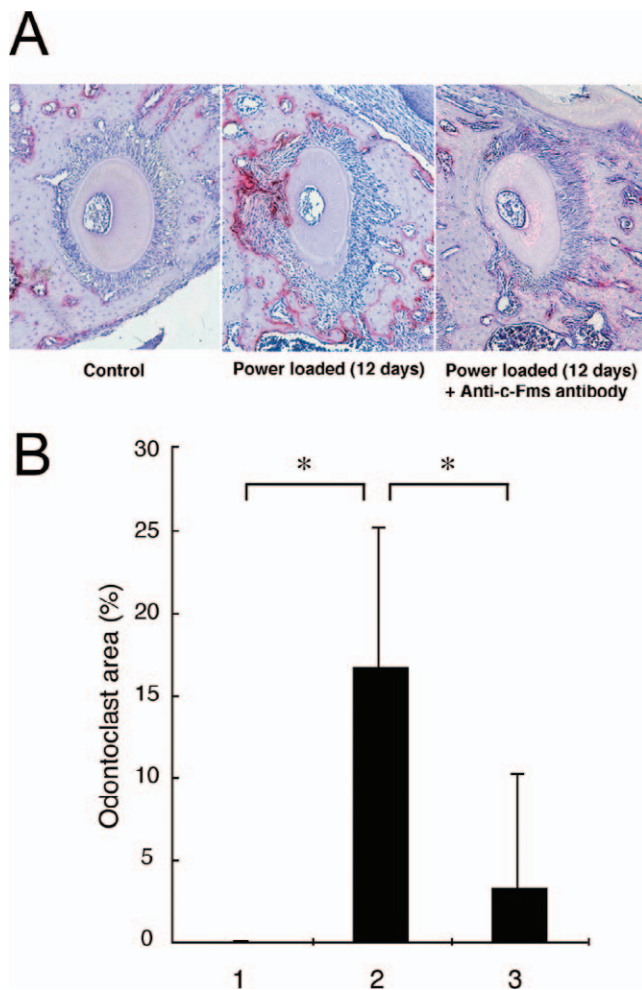


Figure 3. The anti-c-Fms antibody blocks odontoclastogenesis during orthodontic tooth movement (histologic analysis). Mice were injected through daily administration of anti-c-Fms antibody or PBS during orthodontic tooth movements. (A) The histologic picture of the distobuccal root of the first molar after it had been power loaded for 12 days with daily administration of PBS or anti-c-Fms antibody and control. (B) The percentage odontoclast area after the tooth was power loaded for 12 days in control mice (1), mice injected with daily administration of PBS (2), and mice injected with anti-c-Fms antibody (3) during orthodontic tooth movements. Five sections per mouse were measured for resorption area. * $P < .05$ by ANOVA and Scheffe's F-test. Results are expressed as means \pm SD.

stress-induced odontoclastogenesis and root resorption in an orthodontic tooth movement model. We found that the anti-c-Fms antibody inhibited mechanical stress-induced odontoclastogenesis and root resorption. The amount of root resorption strongly differed between control and anti-c-Fms antibody-injected groups on day 12. In our previous study, 200 μ g of anti-c-Fms antibody was injected intraperitoneally into an inflammatory arthritis mouse model, and the osteoclast inhibitory effect was determined to be systemic.¹ In the present study, a lesser amount of anti-c-Fms antibody (10 μ g) was injected into a local site

in an orthodontic tooth movement model. Mechanical stress-induced odontoclastogenesis was inhibited by anti-c-Fms antibody at the local site, even though the amount was small.

Our data indicate that M-CSF might be a therapeutic target in mechanical stress-induced root resorption in orthodontic tooth movement. This suggests that localized anti-c-Fms antibody might be useful for the prevention of root resorption during orthodontic tooth movement. However, in our previous study,² orthodontic tooth movement also was inhibited by same amount of anti-c-Fms antibody. Thus, anti-c-Fms antibody inhibits orthodontic tooth movement at the same time. Additional studies are needed in this area.

M-CSF is concerned with host defense mononuclear phagocytes. Anti-c-Fms antibody prevents pathologic osteoclastogenesis, but the immune-suppressive effects of inhibiting macrophage proliferation and survival coincided. It might be that there is a limitation to M-CSF blockade in clinical applications. In a previous in vitro study, anti-c-Fms antibody prevented RANKL- and M-CSF-induced osteoclast formation at a concentration of at least one order of magnitude less than its capacity to decrease cell number.¹ Results suggested that osteoclast formation is more sensitive to M-CSF inhibition than are macrophage proliferation and survival. The dose of antibody might be important when it is applied in clinical applications. The receptor tyrosine kinase inhibitor SU11248, which prevents activation of the receptor for M-CSF, inhibits osteoclast formation and functioning in vitro and in vivo.³¹ The tyrosine kinase inhibitor imatinib also inhibits c-Fms. It is suggested that there is a possibility of drug treatment for bone destruction due to excessive osteoclast activity.³² However, therapeutic use of M-CSF must be approached with caution, given the significant complications encountered with other forms of anti-cytokine therapy.³³ Additional studies are needed to establish the therapeutic use of M-CSF for the treatment of patients.

CONCLUSION

- M-CSF and/or its receptor is a potential therapeutic target for the treatment of mechanical stress-induced odontoclastogenesis, and injection of an anti-c-Fms antibody might be useful for inhibition of mechanical stress-induced root resorption during orthodontic tooth movement.

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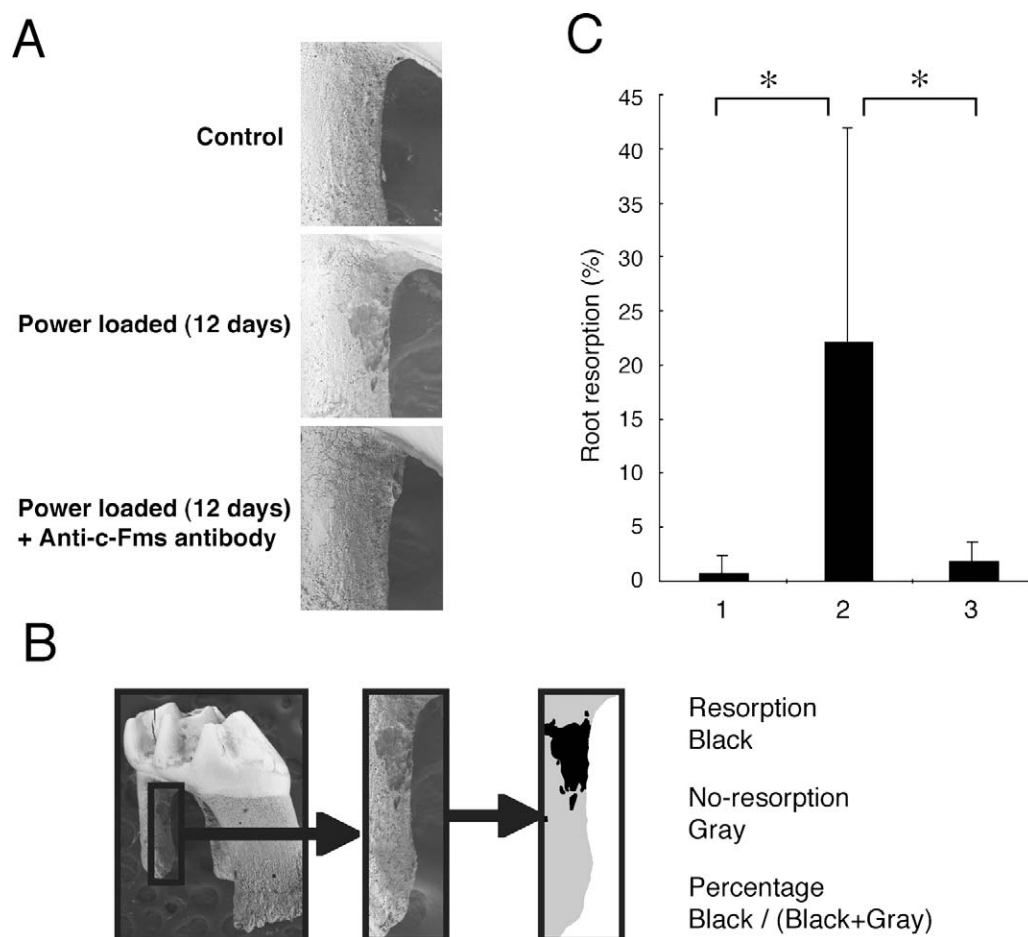


Figure 4. Anti-c-Fms mAb blocks root resorption during orthodontic tooth movement (electron microscopy). Mice were injected through daily administration of anti-c-Fms antibody or PBS during orthodontic tooth movements. (A) The electron microscopic picture of half of the mesial area of the distobuccal root of the first molar after it had been power loaded for 12 days through daily administration of PBS or anti-c-Fms antibody and control. (B) Evaluation of root resorption area on the pressure side of the distobuccal root of the first molar during orthodontic tooth movement by electron microscopy. Gray area: no-resorption area. Black area: resorption area. Root resorption area: percentage of Black area/Black area + Gray area. (C) The percentage of root resorption area after the tooth had been power loaded for 12 days in control mice (1), mice injected with daily administration of PBS (2), or mice injected with anti-c-Fms antibody (3) during orthodontic tooth movements. * $P < .05$ by ANOVA and Scheffe's F-test. Results are expressed as means \pm SD.

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