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Effects of N-acetylcysteine on root resorption after tooth replantation
--Manuscript Draft--

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Corresponding Author:	Koichi Nakamura Hokkaido university Sapporo, Japan
First Author:	Mitsuhiko Nishimi
Order of Authors:	Mitsuhiko Nishimi
	Koichi Nakamura
	Akina Hisada
	Kazuki Endo
	Shuya Ushimura
	Yoshitaka Yoshimura
	Yasutaka Yawaka
Abstract:	In the case of complete luxation with dental trauma, delayed replantation leads to a higher probability of root resorption. We evaluated the efficacy of N-acetylcysteine (NAC) as a root canal medication for trauma-associated root resorption. A rat dental trauma model was used. In the test group, mesial root pulpectomy was carried out after tooth extraction. NAC was applied as a root canal medication followed by replantation. We euthanized the rats after two weeks, excised the maxilla, and prepared thin sections, followed by H-E and TRAP staining. Furthermore, RAW264.7 cells were treated with RANKL and NAC. The effects of NAC on osteoclast differentiation were investigated by determining the numbers of osteoclasts and nuclei and quantifying osteoclast-related mRNA expression levels by real-time PCR. The level of root resorption and number of odontoclasts tended to be lower in the NAC groups than in the control group. In terms of osteoclast differentiation, the total number of osteoclasts was lower in both the 5 and 10 mmol/L NAC groups than in the control group. Real-time PCR revealed that the expression levels of RANK , NFATc1 , DC-STAMP , CD47 , and cathepsin K were lower in the 10 mmol/L NAC group. NAC tended to inhibit root resorption and reduce the number of odontoclasts. NAC also inhibited the expression of osteoclast-related genes. Overall, NAC reduced the number of odontoclasts, inhibited bone resorption, and influenced the inhibition of osteoclast differentiation, fusion, and functions. Therefore, we suggest that NAC may be beneficial in the treatment of root resorption.
Suggested Reviewers:	
Opposed Reviewers:	
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1 INTRODUCTION

Dental trauma takes on complete luxation, in case of the permanent teeth, which occurs

in 1.5% to 16% of trauma cases [1]. The prognosis is relatively favorable if treatment is

carried out within an hour after luxation, but a previous report indicates that replantation

commonly occurs anywhere from 1 to 4 hours after injury [2]. Delaying replantation

results in a poorer prognosis, and research by Guedes *et al.* showed that the incidence of

inflammatory root resorption 1 to 8 years after trauma is 34.34% [3].

If considerable time has elapsed since luxation and the tissue surrounding the root canal

is not expected to survive, a root canal treatment is necessary. Drying of the periodontal

membrane is considered to be the principal cause of trauma-associated root resorption,

but pulp necrosis is also involved [4]. Trauma guidelines published by the International

Association of Dental Traumatology recommend pulp treatment outside the oral cavity

of luxated teeth for which one or more hour(s) has passed since injury [5]. Root canal

treatments, including the treatment of luxated teeth, require anti-inflammatory and

antibacterial effects to fight inflammation occurring at the time of trauma and infections

that develop after luxation, as well as inhibitory effects on root resorption when the

prognosis is poor. An agent that is currently in widespread use is calcium hydroxide

(Ca(OH)$_2$). Calcium hydroxide is used as a root canal medication for traumatized teeth as it has the necessary antibacterial [6], anti-inflammatory [7], hard-tissue-forming [8], and root resorption inhibitory activities [9]. However, leakage of calcium hydroxide outside the apical foramen can lead to complications [10], and reports indicate that its long-term use weakens the root dentin [11], making long-term use problematic. Therefore, the prospect of developing a new root canal medication remains desirable.

The amino acid N-acetylcysteine (NAC) is a glutathione precursor with antioxidant activities in the human body. It has been reported that NAC inhibit a bone resorption due to the decrease of osteoclasts [12]. If NAC shows inhibitory effects on root resorption, it will be possible to justify its use as a novel root canal medication. Unfortunately, there are few reports of its inhibitory effects on root resorption.

A rat traumatized tooth model was prepared to verify the effects of NAC on root resorption in traumatized teeth. The model used the first maxillary molars, and NAC was used as a root canal medication for treating root resorption. In addition to the animal model, we treated RAW264.7 cells with receptor activator of nuclear factor κ-B ligand (RANKL), an osteoclast differentiation factor, and NAC. The effects of NAC on osteoclast differentiation were investigated.

2 MATERIALS AND METHODS

2.1 Preparation of a rat traumatized tooth model

The experimental animals were 6-week-old male SD rats (Sankyo Labo Service

Corporation, Inc. Tokyo, Japan), and NAC (Sigma-Aldrich Corporation, Missouri,

USA) was used as a root canal medication. The study was approved by Hokkaido

University's Animal Committee (protocol approval no.: 14-0063) and was carried out in

accordance with its instructions.

The right and left first maxillary molars were extracted under total anesthesia achieved

using pentobarbital (Somnopentyl®; Kyoritsu Seiyaku Corporation, Tokyo, Japan) and

isoflurane (Escain®; Pfizer, Inc., Tokyo, Japan) and allowed to dry for 1 hour. After

removing the molar caps with a round bur and expanding to no. 25 with a K-file, the

root canal interior was washed with sodium hypochlorite (Neocleaner®; Neo Dental

Chemical Products Co., Ltd. Tokyo, Japan). In the control group, we replanted teeth

after drying without other treatment. The three test groups included treatments with 1, 5,

and 10 mmol/L NAC before replantation of the root canal (n=4). We sealed the apical

end of the root with adhesive resin cement (Super-Bond®; Sun Medical Co., Ltd, Shiga,

Japan) and the coronal end with glass ionomer cement (Fuji Ionomer Type II®; GC,

Tokyo, Japan) before drying. Replantation was carried out, followed by grinding of the

opposing teeth.

The rats were euthanized two weeks later. Perfusion fixation was performed using 4%

paraformaldehyde - maleic acid buffer (Wako Pure Chemical Industries, Ltd., Osaka,

Japan), followed by excision and immersion fixation of the maxilla. After

decalcification with EDTA, we embedded the sample in paraffin by standard reported

methods. We took 5-µm sections perpendicular to the tooth axis. Hematoxylin-eosin (H-

E) staining was carried out, and root sections were photographed. The root section areas

were measured using Image J (NIH, Maryland, USA), and the residual root section area

(%) as a proportion of the mean root section area in untreated teeth was determined for

each treatment group (n = 4). Staining was also carried out with tartrate-resistant acid

phosphatase (TRAP) containing Fast Red Violet LB Salt (Sigma-Aldrich). We used light

microscopy to observe odontoclasts in contact with the root surface.

2.2 Preparation of cells

RAW264.7 cells (ATCC no.: TIB-71^{TM}; Virginia, USA), monocytic macrophages

derived from murine ascites, were used as osteoclast precursor cells. RAW264.7 cells

express RANK, and differentiation to osteoclasts can be achieved by stimulation with

RANKL even without stimulation by a macrophage colony-stimulating factor [13]. The

cells were cultured in Dulbecco's modified Eagle's medium (D-MEM; Wako Pure

Chemical) containing 10% bovine fetal serum (Invitrogen, California, USA) and

66.5 µg/L kanamycin sulfate (Meiji Seika Pharma Co., Ltd., Tokyo, Japan). The cells

were cultured at 37 ℃ in a 5% carbon dioxide atmosphere in a 100-mm standard dish

(BD Falcon, New Jersey, USA), after which they were washed with Dulbecco's

phosphate-buffered saline containing no calcium and magnesium ions (PBS) and

harvested using PBS containing 0.48 mmol/L EDTA.

2.3 Determining the number of viable cells

Cultured RAW264.7 cells were inoculated onto a 96-well plate to a cell density of

1.0×10^4 cells per well. The cells were treated with α-minimum essential medium (α-

MEM: Wako Pure Chemical) containing 10% fetal bovine serum, 434.4 mg/L L-alanyl-

L-glutamine (Wako Pure Chemical), 72.7 mg/L L-ascorbate-2-phosphate (Sigma-

Aldrich), and 66.5 µg/L kanamycin sulfate, to which 100 mmol/L NAC stock solution

(adjusted to pH 7.2) was added to final concentrations of 1, 5, and 10 mmol/L. The

control group was the same but without NAC added. The suspensions were cultured at

37 ℃ in a 5% carbon dioxide atmosphere. We considered the inoculation of RAW264.7

cells onto the plate to be culture day 1. We replaced the culture medium on day 3 and

day 5. We measured the intracellular ATP and determined the viable cell number. The ViaLight™ plus kit (LONZA, Maryland, USA) was used for measurement, and a multi-label counter (Wallac 1420 ARVOSX; PerkinElmer, Inc., Massachusetts, USA) was used to measure fluorescence (n = 8).

2.4 Confirmation of osteoclast differentiation

Cultured RAW264.7 cells were inoculated onto a 24-well plate to a cell density of 1.0×10^4 cells per well. The only substance added was RANKL, to a final concentration of 50 ng/mL in α-MEM in the control group (as used for determining the number of viable cells). Three test groups were also established with NAC concentrations of 1, 5, and 10 mmol/L added at the same time as RANKL. Both the control and test groups were all cultured at 37 ºC under a 5% carbon dioxide atmosphere. The date of inoculation was considered culture day 1, and the culture medium was replaced on day 3. On day 5, the cells were washed with PBS, fixed with 10% formalin, and then washed with distilled water. We stained cells with TRAP stain (pH 5.0) containing Fast Red Violet LB Salt. We determined osteoclast morphology by light microscopy.

2.5 Real-time polymerase chain reaction (PCR)

Cultured RAW264.7 cells were inoculated onto a six-well plate to a cell density of

5.0×10^4 cells per well. For the control group, the only substance added was RANKL,

to a final concentration of 50 ng/mL in α-MEM (as used for determining the number of

viable cells). Three test groups were also set up with NAC at concentrations of 1, 5, and

10 mmol/L added at the same time as RANKL. The control and test groups were

cultured for 12 hours, 24 hours, and 5 days. After culturing, RNA was harvested from

the cells using Trizol (Invitrogen). The reverse transcription reaction was carried out

with Rever Tra Ace-α FSK-101 (Toyobo Co., Ltd., Osaka, Japan), using 1.0 µg of RNA

to synthesize cDNA. We carried out real-time PCR with an ABI7300 PCR system

(Applied Biosystems; Thermo Fisher Scientific, Inc., Massachusetts, USA) using the

following primers (Applied Biosystems; Thermo Fisher Scientific): TRAP

(Mm00475698_m1), RANK (Mm00437132_m1), cathepsin-K (Cath-K;

Mm00484039_m1), NFATc1 (Mm00479445_m1), DC-STAMP (Mm04209236_m1),

OC-STAMP (Mm00512445_m1), and CD47 (Mm00495011_m1). The gene expression

levels of the samples were standardized using the glyceraldehyde 3-phosphate

dehydrogenase expression level and calculated by the $2^{-\Delta\Delta Ct}$ method (n = 4).

2.6 Statistical analyses

All data were expressed as means ± standard deviations. A Bonferroni's test was used to

determine statistical significance, and differences were taken to be significant at

$P < 0.05$.

3 RESULTS

3.1 Histological findings

In untreated teeth, we found calcified material around the root dentin. In the control

group, TRAP staining confirmed existence of many odontoclasts (Fig. 1A). After

replantation with 1 mmol/L NAC application, we found root resorption and

odontoclasts, whereas with 5 and 10 mmol/L NAC, the numbers of both resorption

lacunae and odontoclasts tended to decrease (Fig. 1A). Measurement of the residual root

section area proportions showed a significant difference between the control and

10 mmol/L NAC groups (Fig. 1B).

3.2 Effects of NAC on osteoclast differentiation

We assessed the effects of NAC on osteoclast differentiation. To confirm the presence of

viable cells, NAC was added to RAW264.7 cells at various concentrations, and we

measured intracellular ATP levels after culture for 5 days. Treatments did not show a

decrease in ATP, irrespective of the NAC concentration (Fig. 2A). The number of

osteoclasts was also determined in the control group (RANKL only) and in the test

groups (RANKL+NAC). In the control and 1 mmol/L NAC groups, large osteoclasts

were found, whereas in the 5 and 10 mmol/L NAC groups, the osteoclasts tended to be

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smaller (Fig. 2B). The numbers of osteoclasts with two or more nuclei decreased

significantly in the 5 and 10 mmol/L NAC treatment groups compared with the control

group (Fig. 2C). Comparing the osteoclasts by the number of nuclei, the numbers with

two and three nuclei decreased significantly in the 10 mmol/L NAC group. Similarly,

the numbers with four and five nuclei decreased significantly in the 5 mmol/L NAC

group compared with the control group (Fig. 2D).

3.3 Osteoclast-related gene expression

We performed real-time PCR to quantify osteoclast-related gene mRNA expression. The

group treated with only RANKL was considered the control group, and the groups

treated with each NAC concentration, cultured for 12 hours, 24 hours, and 5 days, were

taken to be the test groups. The mRNA expression after 12 hours decreased significantly

for *DC-STAMP* with 5 and 10 mmol/L NAC and *CD47* with 1 and 5 mmol/L. However

the mRNA expression increased significantly for *NFATc1* with 1 and 5 mmol/L. There

was no significant difference in the others (Fig. 3A). After 24 hours, it decreased

significantly for both *NFATc1* and *DC-STAMP* with 10 mmol/L NAC, but the mRNA

expression increased significantly for *cathepsin K* and *CD47* with 1 mmol/L. There was

no significant difference for *TRAP* and *RANK* (Fig. 3B). After 5 days, the mRNA

expressed decreased significantly for *RANK* with 5 and 10 mmol/L *NAC*, *NFATc1* with

5 mmol/L NAC, *DC-STAMP* with 5 and 10 mmol/L NAC, *CD47* with 5 and 10 mmol/L

NAC, *OC-STAMP* with 5 mmol/L NAC, *cathepsin K* with 5 and 10 mmol/L NAC, and

TRAP with 1 mmol/L NAC (Fig. 3C).

4 DISCUSSION

This study used a rat traumatized tooth model followed by replantation to evaluate the

effects of NAC application on root resorption. This animal model was complemented by

cell culture to address whether the addition of RANKL and NAC to RAW264.7 cells

affected osteoclast differentiation. Luxated teeth have a favorable prognosis after

replantation if drying time is kept short and/or they are kept in an appropriate storage

solution [14, 15]. However, with prolonged drying time and delayed replantation, the

periodontal membrane and dental pulp tissues undergo necrosis, and root resorption

frequently occurs in cases with particularly poor prognoses. The International

Association of Dental Traumatology's guidelines [5] define delayed replantation as

occurring when the drying time is 1 hour or more. Therefore, in this study, the drying

time was set at 1 hour for a traumatized tooth model. Lacunae with odontoclasts were

found as histological signs of resorption in both the control and 1 mmol/L NAC groups.

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The numbers of resorption lacunae and numbers of odontoclasts in contact with the root

tended to decrease in the 5 and 10 mmol/L NAC groups (Fig. 1A). Furthermore, with

respect to the root section area, root resorption was found to be inhibited in the

10 mmol/L NAC group (Fig. 1B). Bacteria and endotoxins derived from the necrotic

pulp tissue may move from dentinal tubules into the gaps and damaged areas left by

root resorption and periodontal membrane necrosis [4]. Therefore, luxation may have

resulted in the periodontal membrane and pulp necrosis, probably resulting in root

resorption in the control group. However, in the 1 mmol/L NAC group, approximately

the same degree of root resorption was found in the control group, irrespective of

pulpectomy and medication application. This result suggests that periodontal membrane

and pulp necrosis are not the sole causes of root resorption and that odontoclast

induction may occur due to mechanical stimulation at the time of luxation. Light

microscopic examination showed a tendency toward inhibition of osteoclast

differentiation in cell cultures with 5 and 10 mmol/L NAC (Fig. 2B), and the total

osteoclast number also decreased in comparison with the control group. In examining

osteoclasts with different numbers of nuclei, two and three nuclei significantly

decreased with 10 mmol/L NAC and four and five nuclei significantly decreased with

5 mmol/L NAC when compared to the control group (Fig. 2C, D, Table 1). NAC treated

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RAW264.7 cells showed no differences in the intracellular ATP levels between the control and any of the NAC concentrations (Fig. 2A). In other words, the decreases in total osteoclast numbers were not due solely to cell death; the inhibition of osteoclast differentiation is likely also involved. A decrease in the number of oligonucleated cells is likely due to inhibition at an early stage in the differentiation process, whereas a decrease in the number of polynucleate cells is due to inhibition of fusion between cells, which occurs later in the differentiation process.

Please insert Table I here.

The cells responsible for root resorption are osteoclasts (i.e., odontoclasts). For tissue resorption, it is important for monocyte/macrophage lineage precursor cells to differentiate into mononucleate osteoclasts and fuse to form polynucleate osteoclasts. Osteoclast differentiation requires the master transcription factor NFATc1 [16] and RANK, RANKL-receptor [17]. DC-STAMP is important [18] for osteoclast fusion. CD47 is involved in the fusion between oligonucleate osteoclasts [19], whereas fusion between polynucleate osteoclasts involves OC-STAMP [20]. In addition, cathepsin K, a cysteine protease that breaks down type-I collagen, is important for bone resorption by osteoclasts [21].

Please insert Table 2 here

In the present study, we found that 10 mmol/L NAC had inhibitory effects on trauma-associated root resorption (Fig. 3; Table 2). In addition, the real-time PCR showed a decreased expression of *RANK*, *NFATc1*, *DC-STAMP,* and *CD47* mRNA with 10 mmol/L NAC. Decreases in *RANK* and *NFATc1* affect osteoclast differentiation, whereas the decreases in *DC-STAMP* and *CD47* affect fusion between osteoclasts. This is consistent with the reductions in oligonucleated cells and numbers of osteoclasts in the present study. The number of osteoclasts with two or more nuclei decreased with 10 mmol/L NAC; the real-time PCR results, however, showed no difference from the control in the expression of *TRAP*, an osteoclast marker gene. *TRAP* is expressed even by mononucleated osteoclasts, but in the present study, two or more nuclei were the criterion for the classification of cells as osteoclasts. This classification may account for the discrepancy between these results. After 5 days, a *OC-STAMP* expression was significantly increased with 10 mmol/L NAC, whereas no significant difference from the control was found in the number of polynucleated cells. This finding is probably due to a decrease in the number of minority nuclei required for fusion of the polynucleated cells. Moreover, at day 5, *cathepsin K* mRNA expression also decreased. For these reasons, osteoclast functions may be inhibited along with osteoclast differentiation and fusion.

In this study, we identified calcified material around the root canal in rats. At many loci, the calcified material was incorporated into the root dentin without a border between the two. Therefore, investigation of the effects of NAC on the root included calcified material. The results of real-time PCR with 5 mmol/L NAC were not markedly different from those with 10 mmol/L NAC, and when comparing root resorption, the 5 mmol/L NAC group was not significantly different from the control group. In cell culture, effects were found without changes in NAC concentration. In the rat model, on applying the root canal medication and sealing both the apical and coronal sides, NAC in the tissues surrounding the root was likely released from only the dentinal tubules. Therefore, it is probable that the observed effects resulted from NAC levels that were, in reality, lower than those relating to the treatment concentrations. In future research, it will be necessary to investigate the appropriate concentrations for root canal medication. The results of this study suggest that in a rat traumatized tooth model, 10 mmol/L NAC can inhibit root resorption by reducing the levels of transcription factors and genes that control osteoclast differentiation and fusion. There have been reports of complications with calcium hydroxide treatment due to apical exudation. Because NAC is an antioxidant amino acid, it is highly probable that it would be absorbed even if apical exudation did occur. In addition, since calcium hydroxide has a pH of 12, it may

establish an alkaline environment for an extended time, which can result in degradation

of root tissues and reduced dentin strength [22, 23]. By contrast, the NAC used in this

study is adjusted to a neutral pH of approximately 7.2 and, therefore, may prevent a

decrease in dentin strength. NAC has antibacterial activities [24, 25] and is reported to

have anti-inflammatory activities due to inhibition of the inflammatory cytokine TNF-α

[7]. Based on these reports, NAC could be used to treat inflammation due to bacterial

infection of the dental pulp or trauma. For that reason, NAC offers one possible option

as a root canal medication for traumatized teeth.

The periodontal tissues of rats and humans have various histological similarities [26]

and, as a result, many dental studies use rats [27]. The results of the present study reveal

a need for further research investigating the comparison with calcium hydroxide, which

is currently widely used for root canal medication, the appropriate NAC concentrations

for root canal medication and the effects of NAC on dentin strength in clinical

application. These should expand into studies on human teeth.

5 CONCLUSIONS

The use of NAC in a rat traumatized tooth model resulted in the inhibition of root

resorption and a tendency toward a decrease in the number of odontoclasts. NAC

application with cell culture resulted in the inhibition of osteoclast-related gene

expression. Several studies report that NAC reduces the number of odontoclasts,

inhibits root resorption, and is involved in inhibiting osteoclast differentiation and

fusion and osteoclast functions. Therefore, there is a potential for the application of

NAC in the treatment of root resorption.

CONFLICTS OF INTEREST

There are no conflicts of interest associated with this study.

ACKNOWLEDGMENTS

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Figure legends

Fig. 1. Tissue images with different NAC concentrations applied to root canal

The first maxillary molars were extracted, pulpectomy was carried out from the coronal

side, and NAC was applied at various concentrations. After drying for 1 hour, replantation

was carried out. A) Calcified material was found around the root canal in untreated teeth.

Resorption lacunae are indicated in the control and 1 mmol/L NAC groups, with fewer in

the 5 and 10 mmol/L NAC groups compared with the control group. Scale bar = 100 μm.

B) Root resorption was inhibited with 10 mmol/L NAC compared with the control group.

Residual root section area (%) = root section area in samples from each test group/

mean root section area in untreated tooth × 100. Error bars represent mean ± SD; n = 4;

$p < 0.01$ (**), $p < 0.05$ (*).

Fig. 2. Effects of NAC on osteoclast differentiation

NAC was added to RAW264.7 cells at various concentrations. After 5 days of culture,

intracellular ATP levels were measured using the ViaLight™ Plus kit, and osteoclasts

were observed with TRAP staining. A) ATP levels decreased in comparison with the

control group (no added NAC) with any added concentration of NAC. Mean ± SD; n = 8.

B) Large osteoclasts were seen in the control and 1 mmol/L NAC groups, whereas cells

trended smaller in the 5 and 10 mmol/L NAC groups (➡: large osteoclasts). Scale

bar=250 μm. C) The total number of osteoclasts (i.e., two or more nuclei) decreased

significantly in the 5 and 10 mmol/L NAC groups. Mean ± SD; n = 4; $p < 0.05$ (*). D)

Classifying osteoclasts by the number of nuclei, osteoclasts with two and three nuclei

decreased significantly in the 10 mmol/L NAC treatment group. The number with four

and five nuclei decreased significantly in the 5 mmol/L NAC group. Mean ± SD; n = 4;

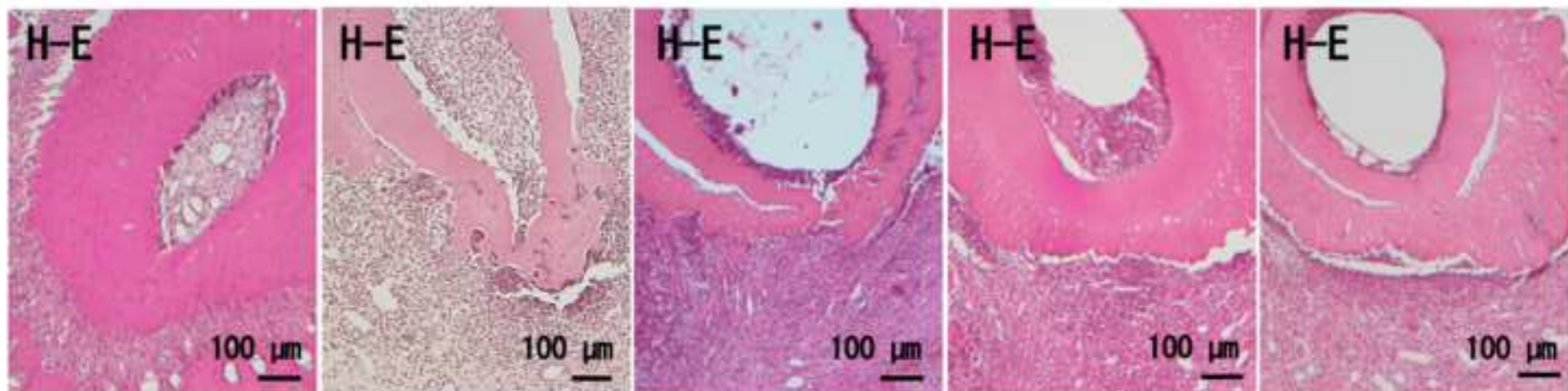p < 0.01 (**); p < 0.05 (*).

Fig. 3. Changes in osteoclast-related gene expression after culture for 12 hours, 24 hours

and 5 days.

RANKL and different concentrations of NAC to RAW264.7 were added to cells, and after

12 hours, 24 hours and 5 days. we compared the mRNA expression levels. A) After 12

hours, compared to control, significant decreases in *DC-STAMP* expression were found

in the 5 and 10 mmol/L NAC groups, and *CD47* expression in the 1 and 5 mmol/L NAC

group. However, significant increase in *NFATc1* expression were found in the 1 and 5

mmol/L NAC group. There was no significant difference in the others. B) After 24 hours,

compared to control, significant decreases were found in *NFATc1* and *DC-STAMP*

expression in the 10 mmol/L NAC group. However, significant increase in *Cath-K* and

CD47 expression were found in the 1 mmol/L NAC group. There was no significant

difference for *TRAP*, *RANK* and *OC-STAMP*. C) After 5 days, compared to control,

significant decreases in expression were found for *TRAP*, *RANK*, *Cath-K*, *DC-STAMP*

and *CD47*, and for *RANK*, *Cath-K, NFATc1*, *DC-STAMP*, *OC-STAMP* and *CD47* in the 5

mmol/L NAC group, and for *RANK*, *Cath-K, DC-STAMP*, *OC-STAMP* and *CD47* in the

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10 mmol/L NAC group.
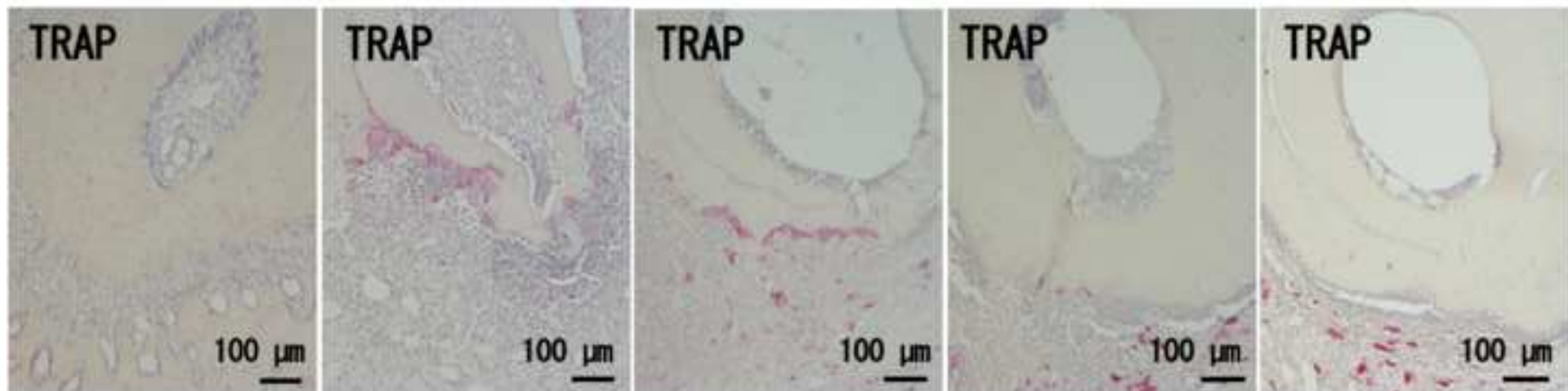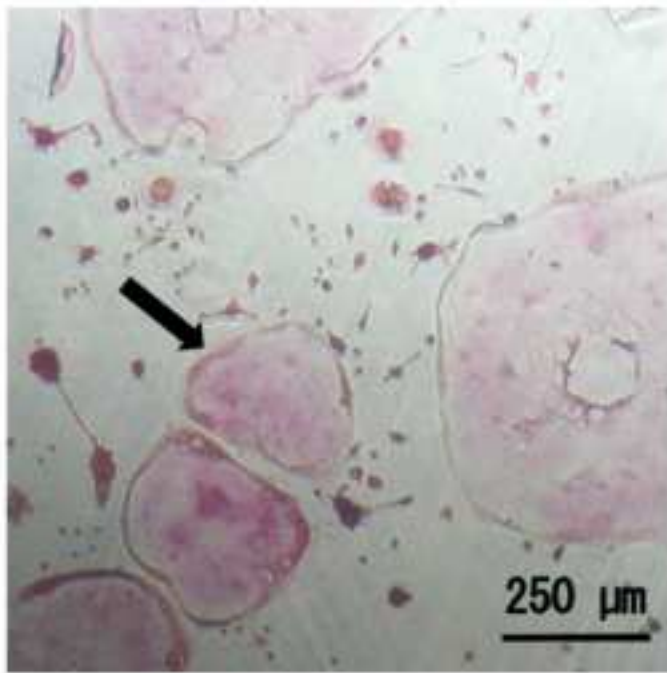
Mean ± SD; n = 4; $p < 0.01$ (**); $p < 0.05$ (*)
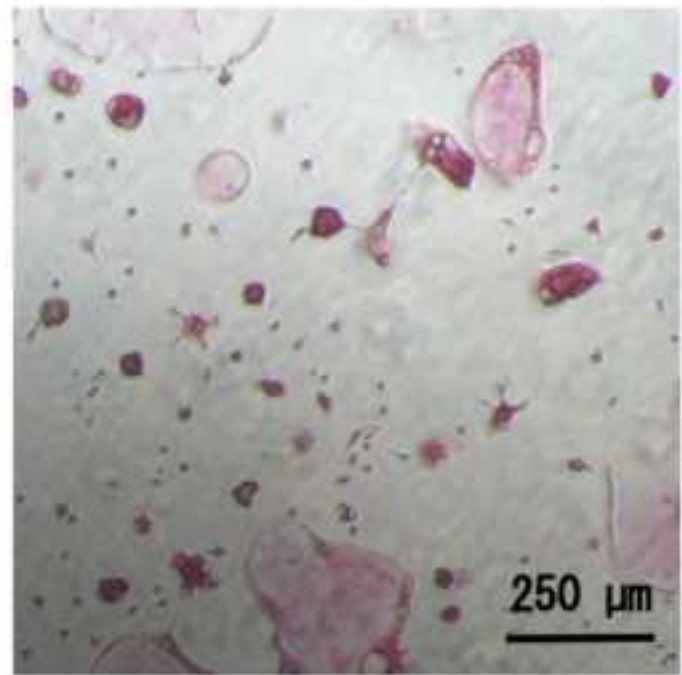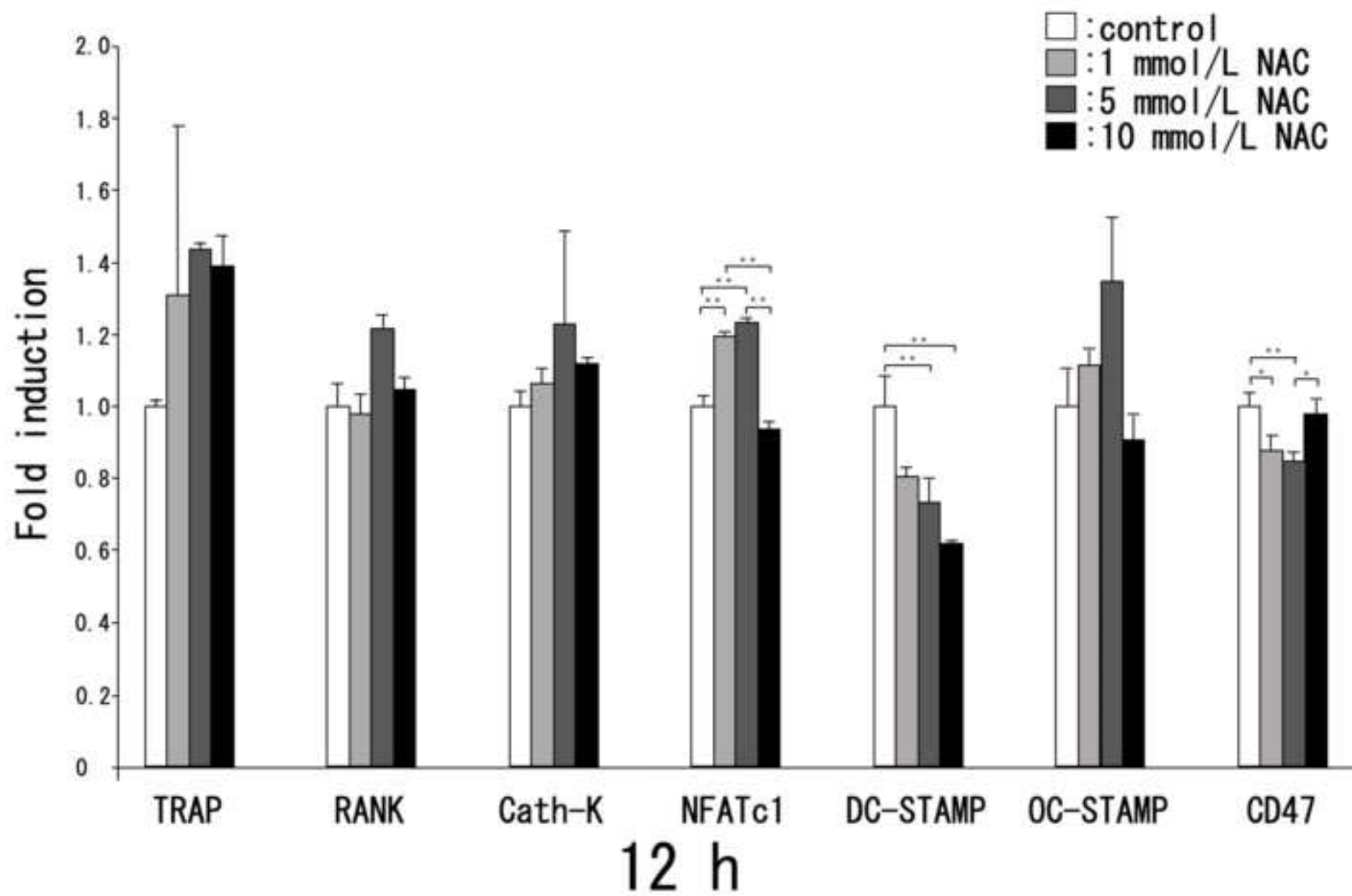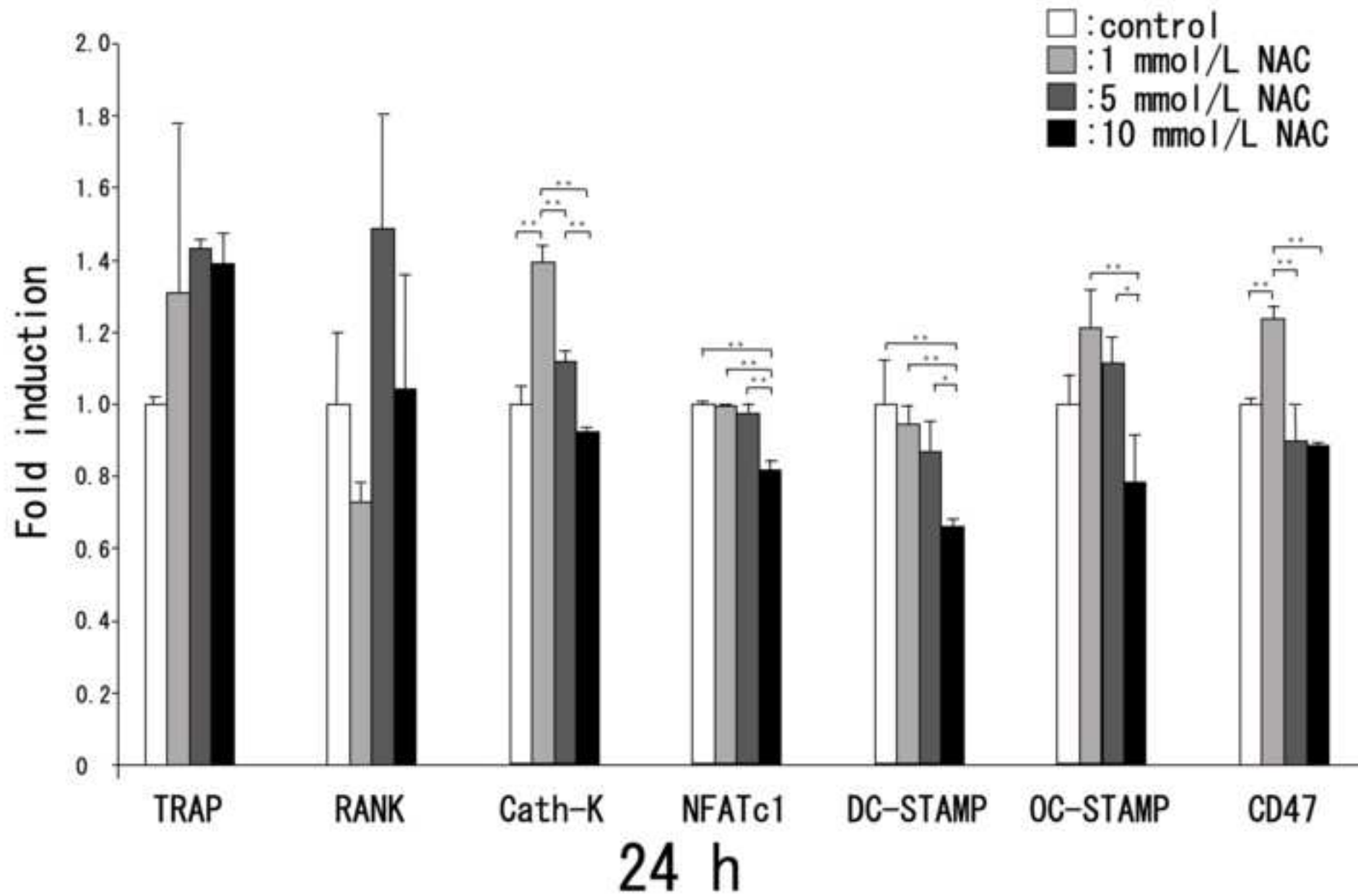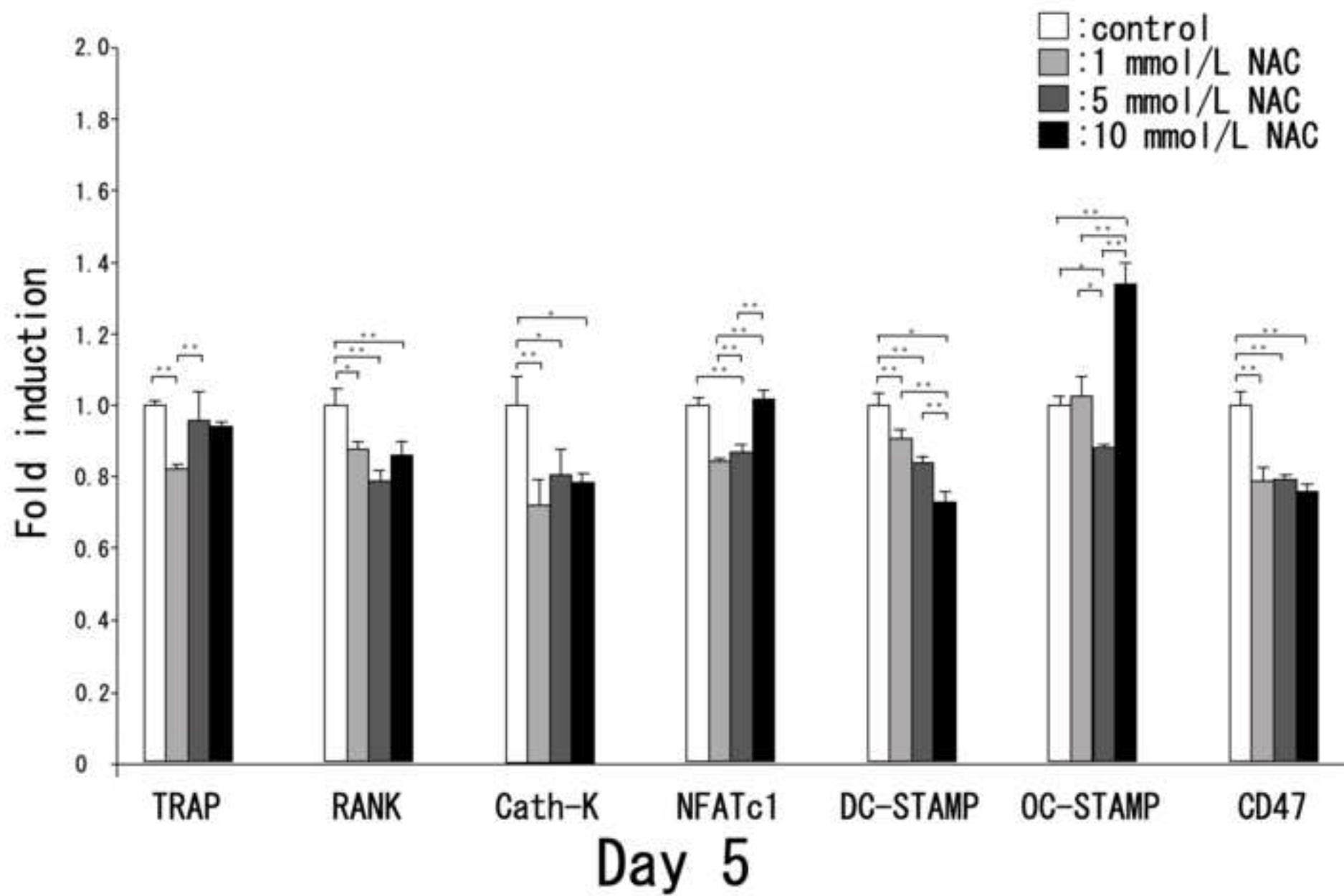
control

1 mmol/L NAC

5 mmol/L NAC

10 mmol/L NAC

12 h

Figure3B — Fold induction of TRAP, RANK, Cath-K, NFATc1, DC-STAMP, OC-STAMP, and CD47 at 24 h under control, 1 mmol/L NAC, 5 mmol/L NAC, and 10 mmol/L NAC.

Day 5
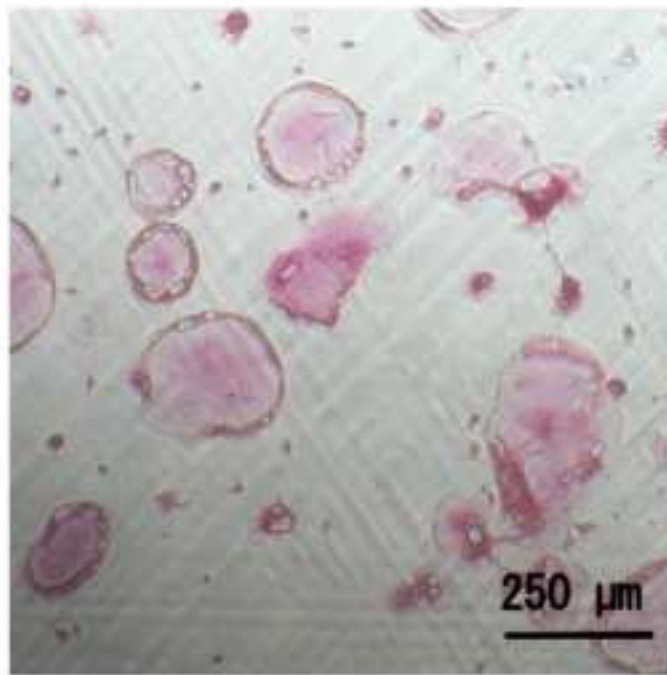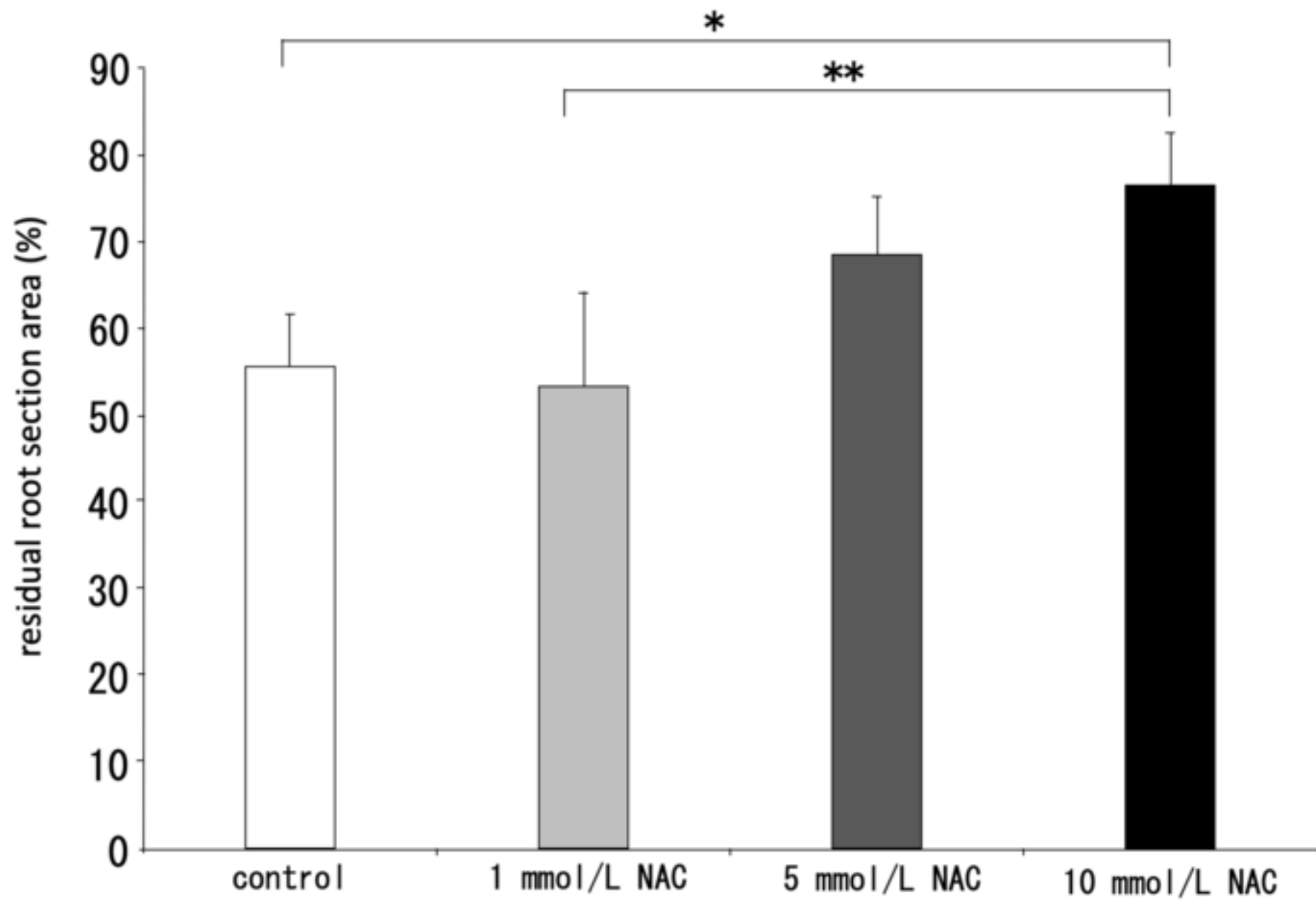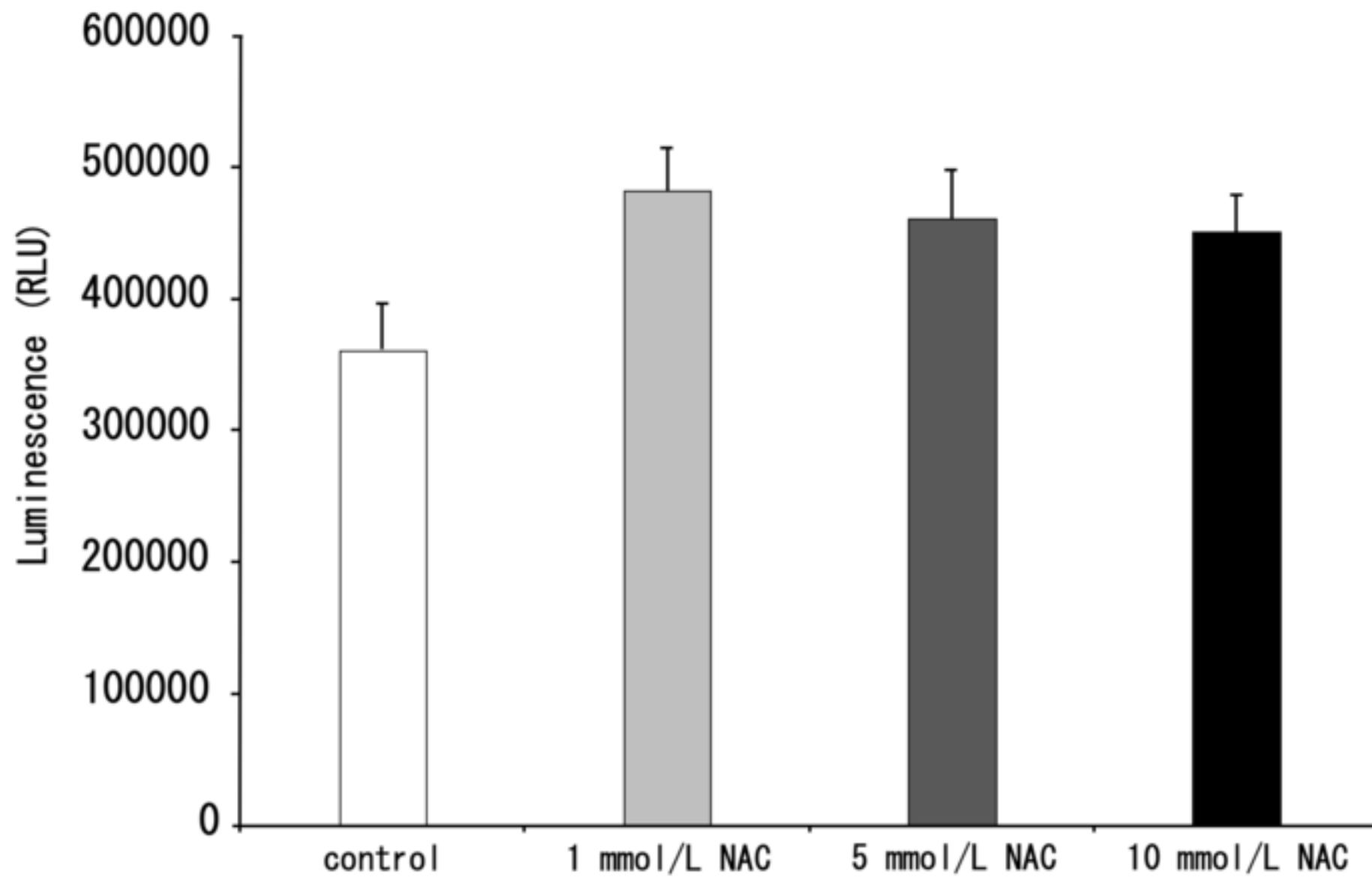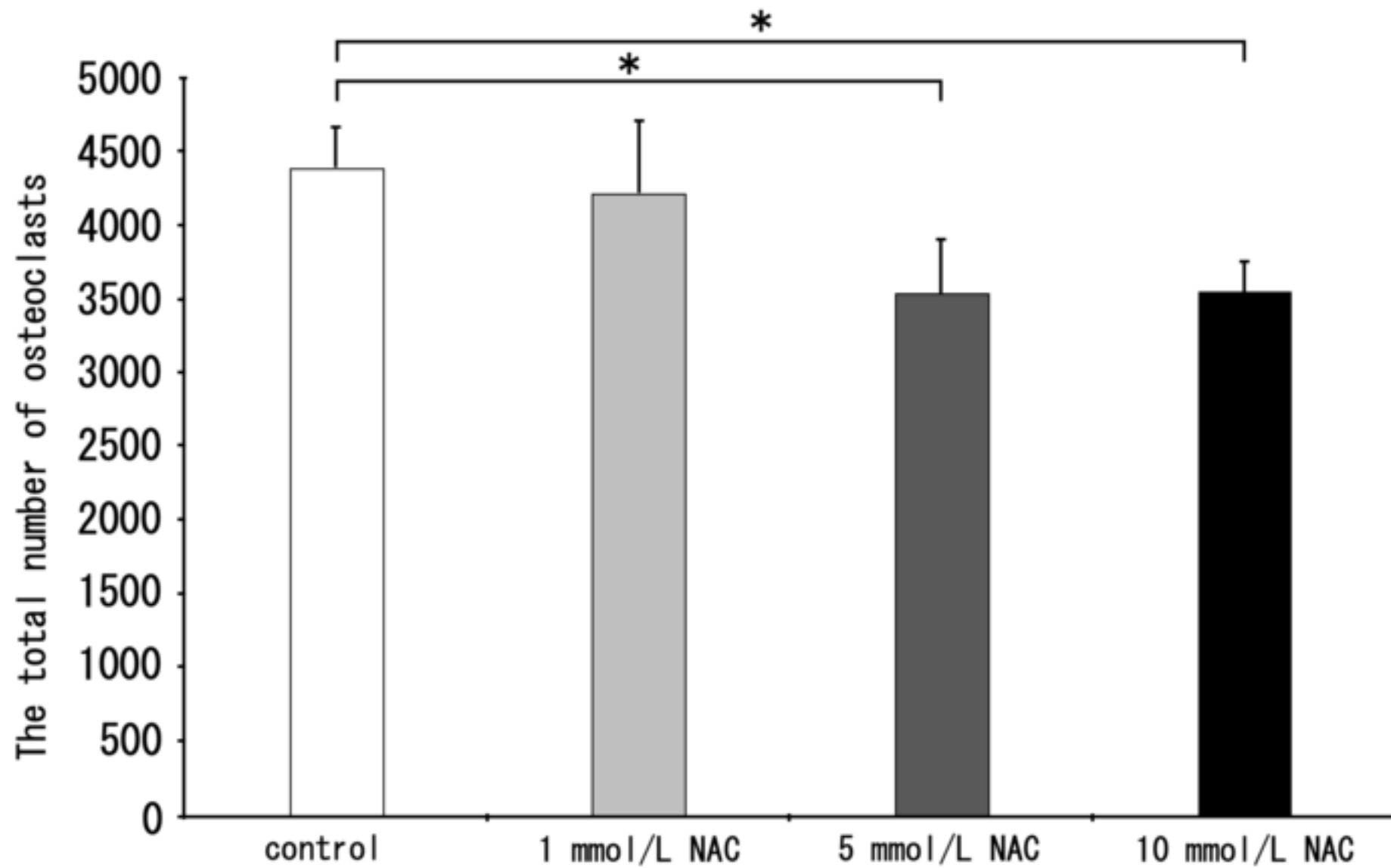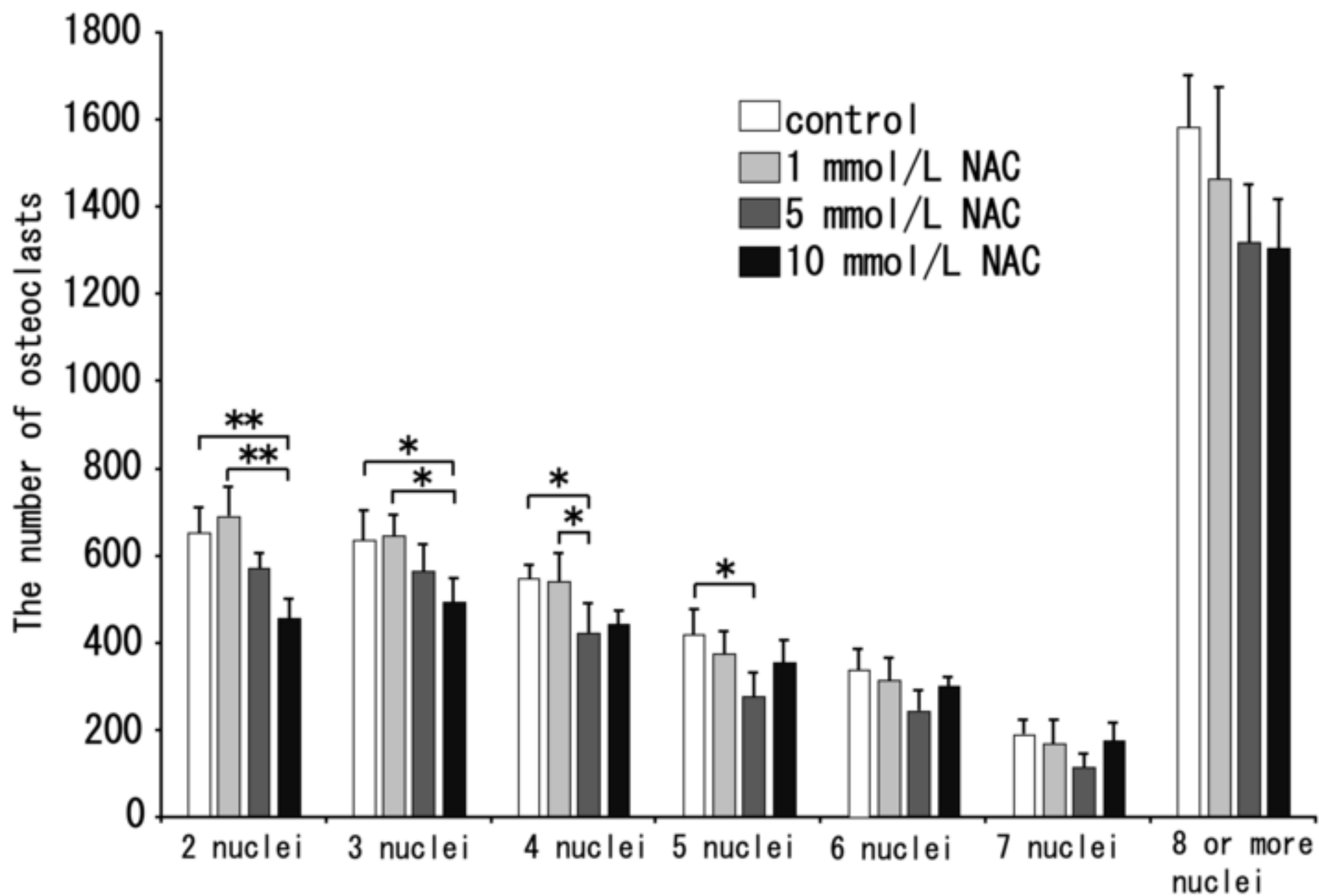
Table 1. Effects of NAC on osteoclast numbers			
	1 mmol/L NAC	**5 mmol/L NAC**	**10 mmol/L NAC**
Number of osteoclasts (two or more nuclei)	—	↓	↓
2 or 3 nuclei	—	—	↓
4 or 5 nuclei	—	↓	—

Table 2. Effects of NAC on osteoclast-related gene expression				
		1 mmol/L NAC	**5 mmol/L NAC**	**10 mmol/L NAC**
12 h	*TRAP*	—	—	—
	RANK	—	—	—
	Cath-K	—	—	—
	NFATc1	↑	↑	—
	DC-STAMP	—	↓	↓
	OC-STAMP	—	—	—
	CD47	↓	↓	—
24 h	*TRAP*	—	—	—
	RANK	—	—	—
	Cath-K	↑	—	—
	NFATc1	—	—	↓
	DC-STAMP	—	—	↓
	OC-STAMP	—	—	—
	CD47	↑	—	—
Day 5	*TRAP*	↓	—	—
	RANK	↓	↓	↓
	Cath-K	↓	↓	↓
	NFATc1	—	↓	—
	DC-STAMP	↓	↓	↓
	OC-STAMP	—	↓	↑
	CD47	↓	↓	↓