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Mechanical stress induced S100A7 expression in human dental pulp cells to augment osteoclast differentiation

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Abstract

Objectives: Mechanical injury of dental pulp leads to root resorption by osteoclasts/odontoclasts. S100 proteins have been demonstrated to be involved in inflammatory processes and bone remodeling. This study aimed to investigate the effect of mechanical stress on S100A7 expression by human dental pulp cells (HDPCs) and the effect of S100A7 proteins on osteoclast differentiation.

Materials and Methods: Isolated HDPCs were stimulated with compressive loading (2 and 6 hr), or shear loading (2, 6, and 16 hr). *S100* mRNA expression and S100A7 protein levels were determined by real-time PCR and ELISA, respectively. Osteoclast differentiation was analyzed using primary human monocytes. The differentiation and activity of osteoclasts were examined by TRAcP staining and dentine resorption. In addition, the expression of S100A7 was analyzed in pulp tissues obtained from orthodontically treated teeth.

Results: Compressive and shear mechanical stress significantly upregulated both mRNA and protein level of S100A7. Dental pulp tissues from orthodontically treated teeth exhibited higher *S100A7* mRNA levels compared to non-treated control teeth. S100A7 promoted osteoclast differentiation by primary human monocytes. Moreover, S100A7 significantly enhanced dentine resorption by these cells.

Conclusions: Mechanical stress induced expression of S100A7 by human dental pulp cells and this may promote root resorption by inducing osteoclast differentiation and activity.

KEYWORDS

dental pulp, inflammation, mechanical stress, Osteoclast, S100 protein, S100A7

1 | INTRODUCTION

Root resorption by osteoclastic or odontoclastic activity may occur primarily in the area ranging from the cervico-apical junction to the root apex (Patel, Kanagasingam, & Pitt Ford, 2009; Patel, Ricucci, Durak, & Tay, 2010). A physiological root resorption is found during primary teeth shedding. However, following pulpal inflammation, root resorption can be occur unwantedly. Various mechanical injuries, such as tooth preparation, orthodontic treatment, dental trauma, and chronic parafunctional force, can lead to pulpal stress and inflammation (Caliskan & Turkun, 1997; Fuss, Tsesis, & Lin, 2003; Patel et al., 2009, 2010). Under conditions of cellular stress and tissue injury, the pulpal cells secrete inflammatory mediators that trigger the recruitment of osteoclast precursor cells, such as monocytes and macrophages, and induce osteoclast differentiation (Iglesias-Linares & Hartsfield, 2017; Rechenberg, Galicia, & Peters, 2016; Udagawa et al., 1990). These events may eventually lead to root resorption.

S100 proteins are calcium-binding proteins that act as regulatory proteins, involved in diverse cellular processes including cell growth, apoptosis, differentiation, cell structure, calcium homeostasis, energy metabolism, inflammation, and migration (Donato et al., 2013). S100 proteins are composed of two EF-hand helix-loop-helices which are linked by a flexible hinge. It has been shown that S100 proteins are upregulated in inflamed dental pulp tissues (Khorasani, Andam-Shahsavari, Zainodini, Khoramdelazad, & Nosratabadi, 2018). An increased S100 expression appears to be correlated with the invasion depth of carious lesion (McLachlan, Sloan, Smith, Landini, & Cooper, 2004). Further, the expression of S100 protein in carious pulp tissue is associated with the expression of inflammatory cytokines (McLachlan et al., 2004).

Several S100 proteins such as S100A7, S100A8, and S100A9 are highly expressed in inflamed tissues and act as endogenous DAMPs (Foell, Wittkowski, Vogl, & Roth, 2007). DAMPs (danger-associated molecular patterns) interact with DAMP-R (DAMP receptor) on immune cells and consequently lead to inflammatory processes including osteoclastogenesis (Manson, Thiemermann, & Brohi, 2012; Patel et al., 2010). The interaction of S100 proteins and DAMP-R, RAGE, and TLR-4 triggers the release of various inflammatory mediators and consequently promotes tissue inflammation (Donato et al., 2013; Rosin & Okusa, 2011).

At present, it is unknown whether mechanical force affects the expression of S100 proteins by dental pulp. Hence, the present study aimed to investigate this by using human dental pulp cells (HDPCs). In addition, the influence of S100 protein on osteoclast differentiation and function was examined.

2 | MATERIALS AND METHODS

2.1 | Cell isolation and culture

HDPCs were obtained from impacted third molars without any pathological conditions from healthy adult subjects. The present study was approved by the Human Research Ethics Committee (HREC-DCU 2017-069) of the Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand. Cell isolation was performed using cell explant technique. Isolated cells were cultured in growth medium consisting of Dulbecco's modified Eagle's medium (GIBCO/Thermo Fisher Scientific, Rochester, NY), fetal bovine serum (10% v/v, GIBCO), Glutamax (2 mM, GIBCO), penicillin (100 U/mL), streptomycin (100 mg/ml, GIBCO), and amphotericin B (0.25 μg/ml, GIBCO). Cells were maintained in a humidified atmosphere with 5% CO2 at 37°C. All experiments were performed with cells from passage 3 to 6 (Satrawaha, Wongkhantee, Pavasant, & Sumrejkanchanakij, 2011).

2.2 | Mechanical force application

HDPCs (350,000 cells/well) were plated in 6-well plates for compressive force application and in 2.5-cm cell culture dishes for shear force application. Cells were "starved" in serum-free culture medium for 3 hr prior to force application. HDPCs were subjected to mechanical loading as previously described (Govitvattana, Osathanon, Taebunpakul, & Pavasant, 2013). Briefly, mechanical stress was loaded onto HDPCs by placing a plastic cylinder with metal coins. The mechanical load was calculated as the formula: total weight (weight of coins, plastic cylinder, and media)/area of the tissue culture well. The shear force was produced by a newly designed Cell Shear Force Loading Apparatus Using Angular Flow of Fluids (Appendix S1 and Figure S1 and S2). The cells and supernatant were collected for assessment of mRNA and protein levels at 2, 6, and 16 hr after mechanical loading. For the controls, cells were cultured in normal tissue culture without the force application.

2.3 | Cell viability assay

HDPCs were subjected to 2 g/cm^2 of compressive force for 6 hr or with 0.2 Pa shear force for 16 hr. Cells maintained in normal culture condition without mechanical loading were used as controls. MTT assays were used to determine cell viability. Briefly, cells were incubated with MTT solution and subsequently formazan crystals were solubilized in dimethyl sulfoxide/glycine buffer at pH 10. The absorbance was measured at 570 nm using a microplate reader (ELx800; BIO-TEK®, VT, USA).

2.4 | Collection of dental pulp tissues from orthodontically moved teeth

Premolar teeth were obtained from orthodontic patients before or following approximately 4 months of orthodontic tooth movement. We selected this time point based on the treatment plan of the patients. The protocol was approved by the Human Research Ethics Committee (HREC-DCU 2017-078) of the Faculty of Dentistry, Chulalongkorn University. Previous reports demonstrated that an inflammatory response of dental pulp tissues and root resorption occurred after an orthodontic tooth movement of about 3 weeks and 7 weeks (Lazzaretti et al., 2014; Owman-Moll, Kurol, & Lundgren, 1996). Dental pulp tissues were collected, minced, and immersed in

TABLE 1 Primer sequences for qPCR

Gene name	Sequence ID	Primer sequences (5' to 3')		Product size
IL1β	NM_000576.2	F	GCAGAAGTACCTGAGCTCGC	174
		R	CTTGCTGTAGTGGTGGTCGG	
IL6	NM_000600.4[a]	F	ATGCAATAACCACCCCTGAC	110
		R	AAAGCTGCGCAGAATGAGAT	
VEGF	NM_001025366.2[a]	F	ATGAGGACACCGGCTCTGACCA	126
		R	AGGCTCCTGAATCTTCCAGGCA	
S100A4	NM_002961.2[a]	F	GAACTAAAGGAGCTGCTGACCC	60
		R	TTCATCTGTCCTTTTCCCCAA	
S100A7	NM_002963.3	F	GATTGACAAGCCAAGCCTGC	101
		R	CAAAGACGTCGGCGAGGTAA	
S100A8	NM_001319196.1[a]	F	AAGCTGTCTCTGATGGCCTG	160
		R	GTCAACATGATGCCCACGGA	
RAGE	NM_001136.4[a]	F	CCAACTACCGAGTCCGTGTC	72
		R	CCGTGAGTTCAGAGGCAGAA	
RANK	NM_003839.3[a]	F	CTGTGGCCCGGATGAATACT	85
		R	CAGGGCCTTGCCTGTATCAC	
NFATc1	NM_172390.2[a]	F	GCTGCATGGCTACTTGGAGA	159
		R	TTGGTGTTGGAGAGGATGGC	
CTSK	NM_000396.3	F	AGGCAGCTAAATGCAGAGGG	119
		R	GAAGGAGGTCAGGCTTGCAT	
CCL2	NM_002982.3	F	CCCCAGTCACCTGCTGTTAT	171
		R	TGGAATCCTGAACCCACTTC	
GAPDH	NM_002046.6	F	CACTGCCAACGTGTCAGTGGTG	121
		R	GTAGCCCAGGATGCCCTTGAG	

[a]The gene has transcript variants of mRNA, and the primer binds to all transcript variants.

TRIzol™ reagent (Invitrogen, Thermo Fisher Scientific), and RNA was isolated. Dental pulp tissues from teeth without orthodontic force application were used as controls.

2.5 | Real-time quantitative polymerase chain reaction (qPCR)

TRIzol™ reagent was used for total RNA isolation (Invitrogen, Thermo Fisher Scientific). Subsequently, complementary DNA was obtained by using reverse transcriptase (iScript™ Reverse Transcription Supermix, Bio-Rad, Hercules, CA). Subsequently, qPCR was performed using the SYBR detection system (iTaq™ Universal SYBR® Green Supermix, Bio-Rad) in a LightCycler® 480 II (Roche, Basel, Switzerland). The expression was normalized to *GAPDH* expression values. Relative mRNA was determined by using the Ct method. The primer sequences are shown in Table 1.

2.6 | Enzyme-linked immunosorbent assay

The level of S100A7 protein in culture supernatant was determined using S100A7 ELISA kits (CircuLex, MBL, Nagoya, Japan) according to the manufacturer's protocol. The absorbance was measured at 450 nm using a microplate reader.

2.7 | Isolation of human monocytes and generation of osteoclasts

Whole blood was obtained from healthy volunteers. The human blood collection procedure was approved by the Human Research Ethics Committee (HREC-DCU 2017-069) of the Faculty of Dentistry, Chulalongkorn University. Peripheral blood mononuclear cells (PBMCs) were isolated using density centrifugation (Histoplaque, Sigma-Aldrich, St. Louis, MO), and $CD14^+$ monocytes were enriched using human CD14 microbeads (Miltenyi Biotec, Auburn, CA). Cells were cultured in osteoclast-conditioned medium consisting of MEM alpha modification medium (HyClone, Logan, UT), certified FBS (10% v/v, GIBCO), Glutamax (0.2 mM), penicillin (100 U/mL), streptomycin (100 mg/ml), amphotericin B (0.25 μg/ml), recombinant human (rh) RANKL (25 ng/ml, (Peprotech, Rocky Hill, NJ), and rhM-CSF (25 ng/ml, Peprotech). Cells were incubated in a humidified atmosphere with 5% CO2 at 37°C. Culture media were replaced every 3 days.

2.8 | Determination of osteoclast number

$CD14^+$ monocytes (100,000 cells/well) were plated in 96-well plates. Cells were maintained in 200 μl of osteoclastogenesis-inducing medium. In the experimental condition, recombinant human S100A7

(Circulex, MBL International, Woburn, MA) (1, 10, and 100 ng/ml) was added to the culture medium. At day 14, the presence of osteoclasts was assessed by staining the cells for TRAcP (tartrate-resistant acid phosphatase) activity as previously described (Susa, Luong-Nguyen, Cappellen, Zamurovic, & Gamse, 2004). Subsequently, cells were observed under a bright field microscopy (Olympus BX50, Tokyo, Japan) and imaging processor (Olympus DP72) with 100x stage objectives. Cells that positively stained for TRAcP activity and contained 3 nuclei were counted from three fields per sample. Monocytes cultured in osteoclastogenesis-inducing medium without S100A7 were used as controls. Fold changes in osteoclast number were calculated as the ratio of the difference of the osteoclast number from S100A7 treatment and untreated controls.

2.9 | Gene expression by osteoclasts

$CD14^+$ monocytes (300,000 cells/well) were plated in 48-well plates and maintained in 500 µl of osteoclast-conditioned medium with or without rhS100A7 for 4 and 7 days. The mRNA expression of *NFATc1*, *CTSK* (cathepsin K), *RAGE*, *RANK*, and *CCL2* was determined using qPCR as described above.

2.10 | Pit resorption assay

$CD14^+$ monocytes (200,000 cells/well) were seeded on 7-mm-diameter dentine slices in 48-well plates. Cells were cultured in osteoclastogenesis-inducing medium in the presence or absence of S100A7 (1 and 100 ng/ml). At day 14, cells on the dentin slices were removed by sonication in 25% ammonium hydroxide. Specimens were stained with 1 mg/ml toluidine blue. Resorption pits were visualized under a bright field microscope equipped with an imaging processor with 100x stage objectives. The micrographs were taken from three fields of each dentin slice, and the area of resorption was measured using a polygonal measurement tool of CellSens software (Olympus). The monocytes cultured in osteoclastogenesis-inducing medium were used as controls. Fold change in area of resorption pits was calculated as the ratio of the difference of the pits following S100A7 incubation and the control.

2.11 | Statistical analysis

All data were expressed as mean ± standard deviation. Data were obtained from at least two independent experiments. Statistically

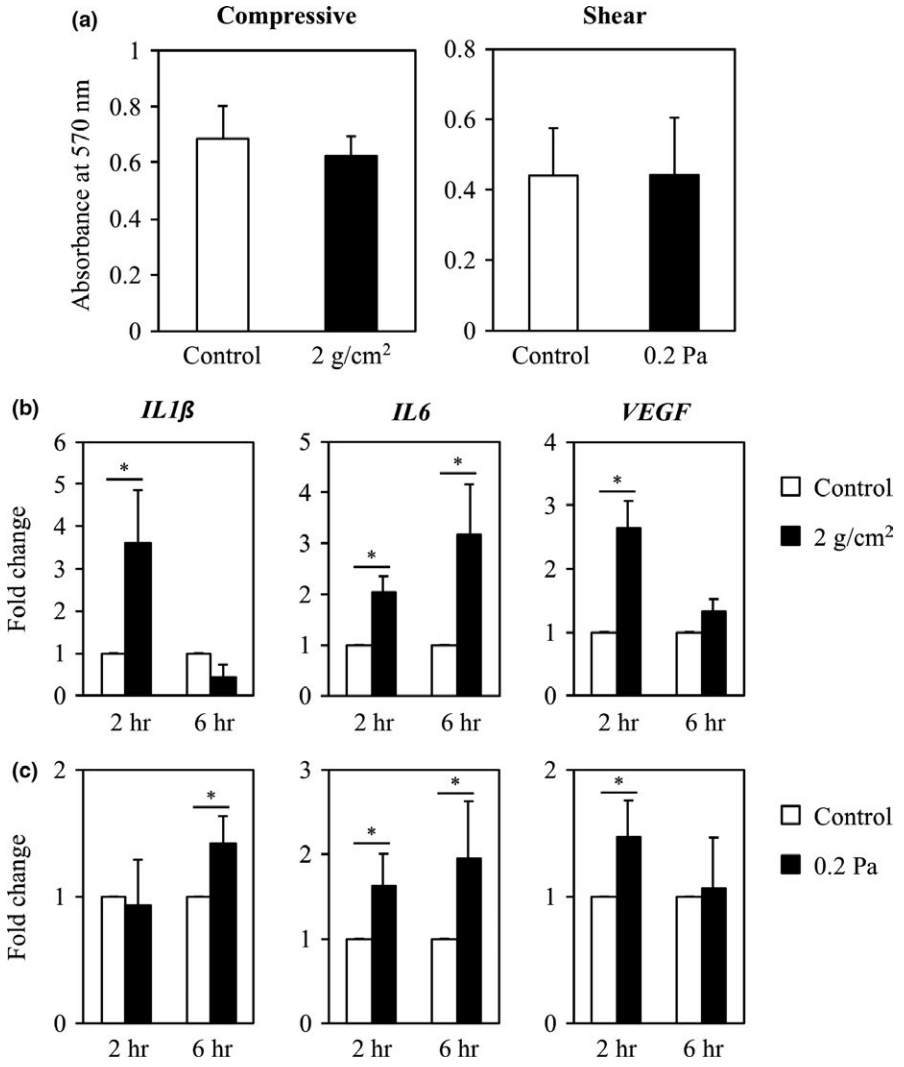

FIGURE 1 Effect of mechanical stress on cell viability and inflammatory gene expression in vitro. (a) HDPCs were subjected to a compressive loading (2 g/cm^2) or to shear loading (0.2 Pa) for 6 hr, and cell viability was determined by MTT assays; $N = 4$. HDPCs subjected to (a) compressive loading, or (b) shear loading for 2 and 6 hr, and the expression of the inflammatory genes, *IL1B*, *IL6*, and *VEGF*, was assessed by using qPCR; $N = 3$. Data are expressed as mean ± standard deviation. $^*p < 0.05$

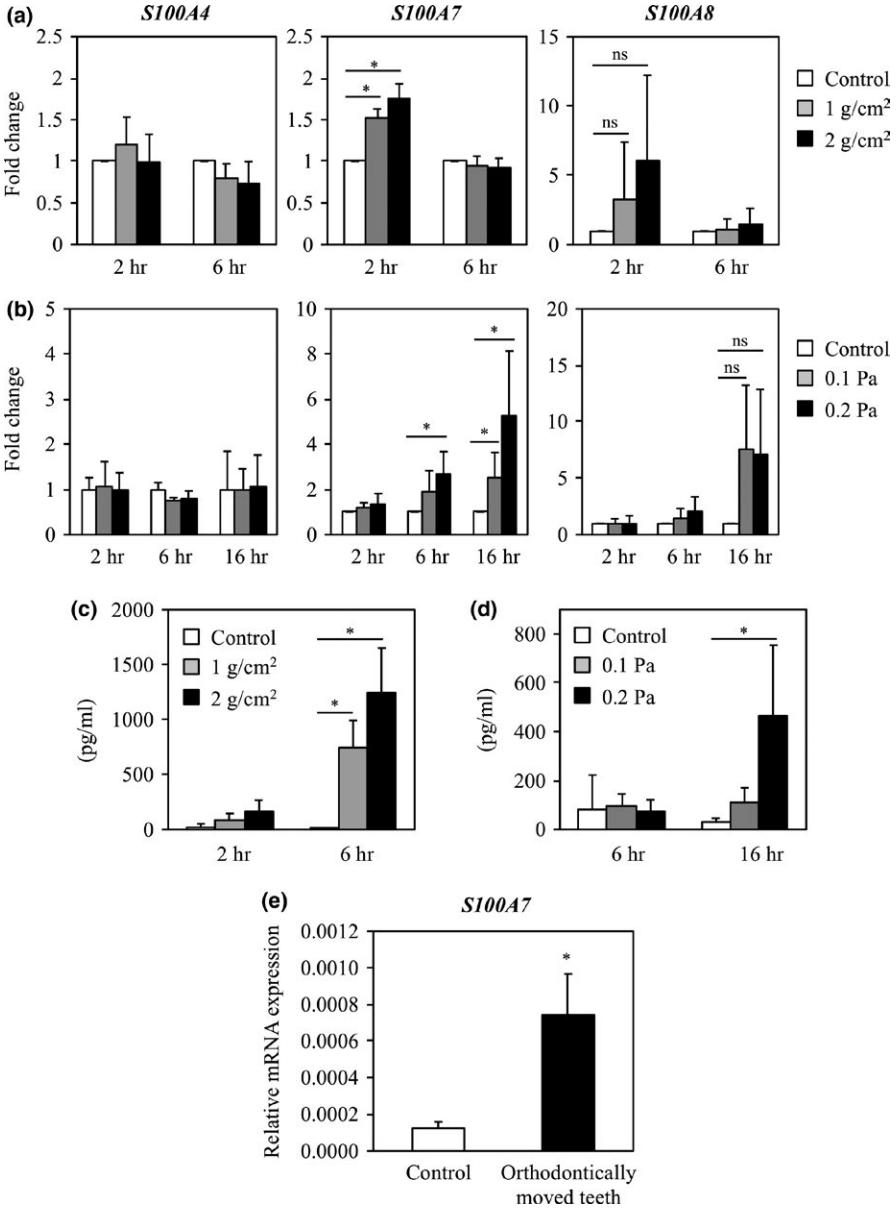

FIGURE 2 Effect of mechanical stress on *S100* expression. HDPCs were subjected to (a) compressive loading for 2 and 6 hr, or (b) shear loading for 2, 6, and 16 hr. mRNA expression of *S100A4*, *S100A7*, and *S100A8* was determined by qPCR; *N* = 4. (c) S100A7 protein levels in the culture supernatant of (c) HPDCs subjected to compressive loading (1–2 g/cm^2) or (d) shear loading (0.1–0.2 Pa) were determined by ELISA; *N* = 4. (e) Dental pulp tissues were collected from orthodontically moved teeth and untreated control teeth. The *S100A7* mRNA expression was determined by qPCR; *N* = 6. Data are expressed as mean ± standard deviation. ns, no significant difference. *$p < 0.05$

significant differences were determined using Student's *t* test. A statistically significant difference was considered when *p* value <0.05.

3 | RESULTS

3.1 | In vitro effect of mechanical stress on cell viability and inflammatory response in HDPCs

To characterize the HDPCs used in this study, the surface expression of the immune progenitor marker, CD34, the leukocyte marker, CD45, and the fibroblast markers, CD44, CD90, and CD105, were assessed by flow cytometric analyses. The data demonstrated that the HDPCs were negative for the markers of immune progenitors and leukocytes, but the cells were highly positive for the fibroblast markers, CD44, CD90, and CD105 (Figure S3). To evaluate the in vitro mechanical stress model used in this study, cell viability and expression of inflammatory mediators were determined. The mechanical loading, compressive and shear force, did not affect HDPC's viability (Figure 1a), while the forces significantly induced expression of the inflammatory mediators, *IL1β*, *IL6*, and *VEGF* by HDPCs (Figure 1b,c).

3.2 | Mechanical stress induced S100A7 expression

We next investigated the effect of mechanical stress on the expression of S100 genes in HDPCs. The compressive loading significantly induced *S100A7* mRNA expression at 2 hr (Figure 2a), and the shear loading significantly induced the expression of *S100A7* at 6 and 16 hr (Figure 2b). Meanwhile, no significant change was noted in the mRNA expression of *S100A4* and *S100A8* when HDPCs were subjected to either compressive or shear loading (Figure 2a,b). In contrast to HDPCs, human periodontal ligament

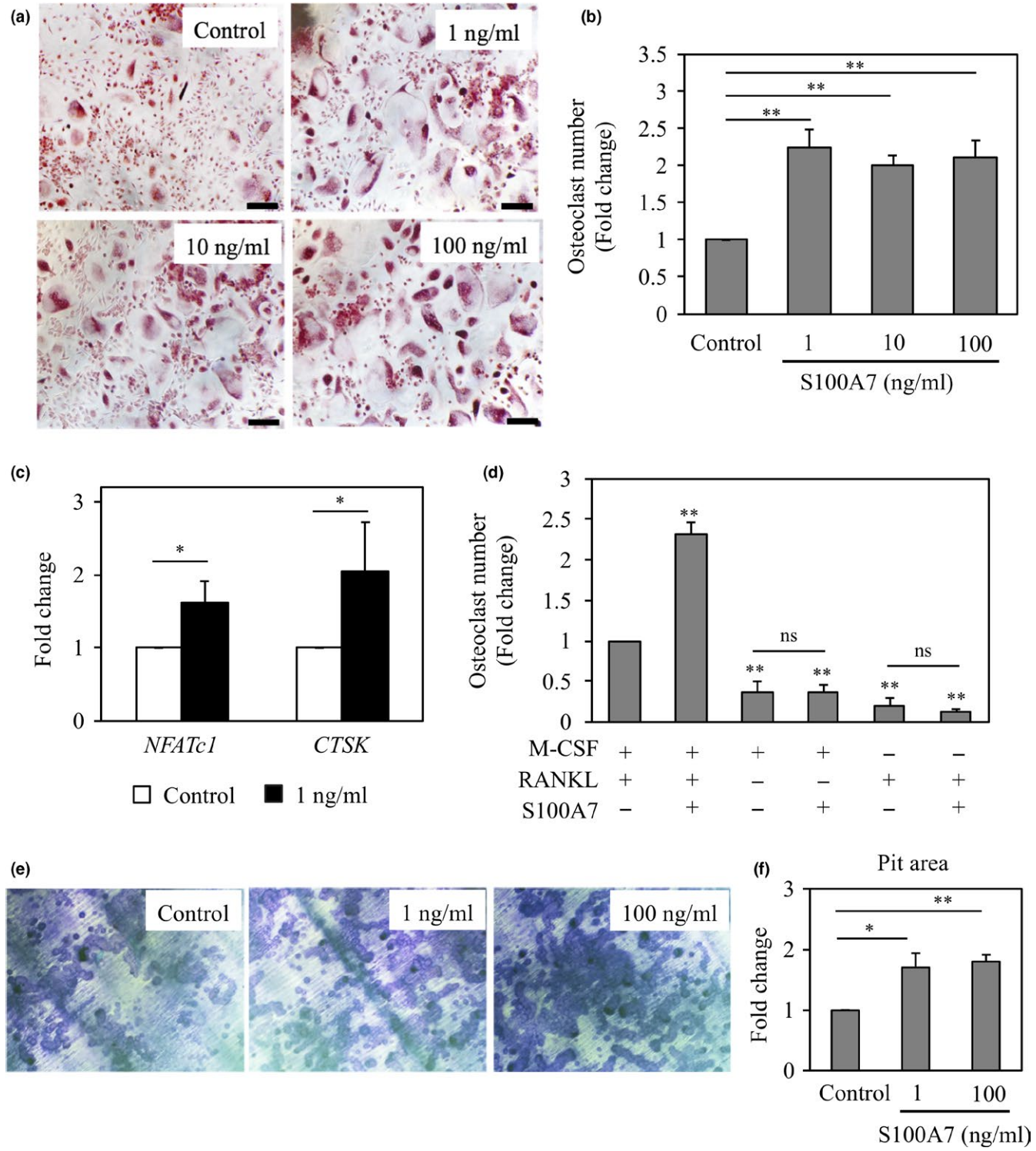

FIGURE 3 Osteoclast differentiation in the presence of S100A7. Human CD14$^+$ monocytes were differentiated to osteoclasts in the presence of S100A7 at the concentration ranging from 1 to 100 ng/ml. (a) Representative images of TRAcP staining of osteoclasts after 14 days of differentiation. (b) TRAcP-positive cells containing 3 nuclei were identified as osteoclasts. (c) Human CD14$^+$ monocytes were differentiated to osteoclasts in the absence or presence of S100A7 (1 ng/ml), and the expression of *NFATc1* and *CTSK* was determined at day 4 of differentiation by qPCR; $N = 3$. (d) Human CD14$^+$ monocytes were differentiated to osteoclasts in the absence or presence of S100A7 (100 ng/ml), *M*-CSF, and RANKL; $N = 4$. (e) Representative images of resorption pits in the presence of S100A7 at 1 ng/ml and 100 ng/ml. (f) The resorption pit area from osteoclast cultures in the presence of S100A7 at 1 ng/ml and 100 ng/ml; $N = 3$. Data are expressed as mean ± *SD*. $^*p < 0.05$

cells (HPDLs) showed no upregulation of *S100A7* or *S100A4* in response to compressive loading, while a significant upregulation of *S100A8* was observed (Figure S4). The upregulation of mRNA expression of S100A7 was confirmed at the protein level by ELISA. S100A7 protein levels were notably increased after treatment with compressive and shear stress for 6 and 16 hr, respectively (Figure 2c,d). The data indicate that of the different S100 proteins S100A7 was preferentially induced by HPDCs in response to mechanical stress.

The mRNA expression of *S100A7* in dental pulp was also observed in tissues obtained from orthodontically treated premolars. Results demonstrated that the pulp tissues of these teeth exhibited a significantly higher mRNA expression of *S100A7* (Figure 2e).

As we observed an upregulation of *IL1ß*, *IL6*, and *VEGF* expression in conjunction with an increased expression of S100A7 by HDPCs subjected to the mechanical stress, we subsequently investigated whether the pro-inflammatory cytokines affected the expression of S100A7. HPDCs were treated with various concentrations of IL-1 and IL-6, and the protein level of S100A7 was assessed (Figure S5). We found that IL-1, but not IL-6, enhanced S100A7 production (Figure S5a,b). In addition, HPDCs were also treated with various concentrations of S100A7, and the expression of *IL1ß*, *IL6*, and *VEGF* was determined. S100A7 did not influence the expression of *IL1B*, *IL6*, and *VEGF* (Figure S5c). These results suggest that the upregulation of S100A7 by HDPCs could have been caused by IL-1 derived from the mechanical stress-stimulated HDPCs.

3.3 | Effect of S100A7 on osteoclast differentiation and activity

As S100A7 was found to be secreted by HDPCs subjected to mechanical stress, its effect on osteoclast differentiation was examined. Human $CD14^+$ monocytes were isolated and osteoclastogenesis was induced by *M*-CSF and RANKL in the absence or presence of S100A7 in concentrations ranging from 1 to 100 ng/ml. $TRAcP^+$ multinucleated cells were subsequently identified (Figure 3a). Osteoclast formation and number were significantly increased in the presence of S100A7. This was found with all concentrations of S100A7 tested (Figure 3a,b). Osteoclast marker genes, *NFATc1* and *CTSK*, were also upregulated in the presence of S100A7 (Figure 3c). To further investigate the influence of S100A7 on osteoclast induction, osteoclast differentiation was induced in the culture medium with or without *M*-CSF or RANKL (Figure 3d and S6). Human monocytes cultured with both *M*-CSF and RANKL were used as the positive control. In the condition that the monocytes were cultured with either *M*-CSF or with RANKL, no osteoclasts were generated. Also, the addition of S100A7 to these cultures had no effect on the formation of osteoclasts. However, S100A7 promoted significantly osteoclast differentiation in the presence of both *M*-CSF and RANKL.

Next, the effect of S100A7 on resorption by osteoclasts was examined. Human $CD14^+$ monocytes were seeded on dentine slices. Osteoclast differentiation was induced by *M*-CSF and RANKL, and these cultures were supplemented with 1 and 100 ng/ml of S100A7. S100A7 was found to induce more resorption of dentine slices (Figure 3e); a significantly increased pit area was apparent (Figure 3f). In addition, S100A7 upregulated *RAGE* but not *RANK* mRNA expression by osteoclasts (Figure 4). *CCL2*, a chemokine that possesses a stimulatory effect on osteoclasts (Khan, Hashimi, Bakr, Forwood, & Morrison, 2016; Miyamoto et al., 2009) as well as being the target gene of RAGE activation (Gu et al., 2006; Tesch, 2008), was also upregulated in a way comparable to RAGE (Figure 4c) supporting the possibility that RAGE may be involved in S100A7-enhanced osteoclastogenesis. Our results indicate that S100A7 promotes osteoclast differentiation and activity.

4 | DISCUSSION

Mechanical injuries cause an inflammation of dental pulp tissues, which consequently may lead to root resorption. Several findings support the option that root resorption can be triggered by factors expressed in the inflamed dental pulp (Bastos et al., 2014; Mattison, Gholston, & Boyd, 1983; Mincik, Urban, & Timkova, 2016; Wedenberg & Zetterqvist, 1987). A previous report has shown that HDPCs and HPDLs are involved in osteoclast differentiation (Uchiyama et al., 2009). During inflammation, locally present immune cells and other cell types release chemokines and pro-inflammatory cytokines to recruit osteoclast precursor cells such as monocytes and macrophages (Rechenberg et al., 2016; Udagawa et al., 1990). This is followed by an induction of osteoclast/odontoclast differentiation (Edwards & Mundy, 2011; Silva, Garlet, Fukada, Silva, & Cunha, 2007; Takayanagi, 2010). Several studies have shown the presence of osteoclasts/odontoclasts in the resorption area (Patel et al., 2010; Wedenberg & Lindskog, 1985; Wedenberg & Zetterqvist,

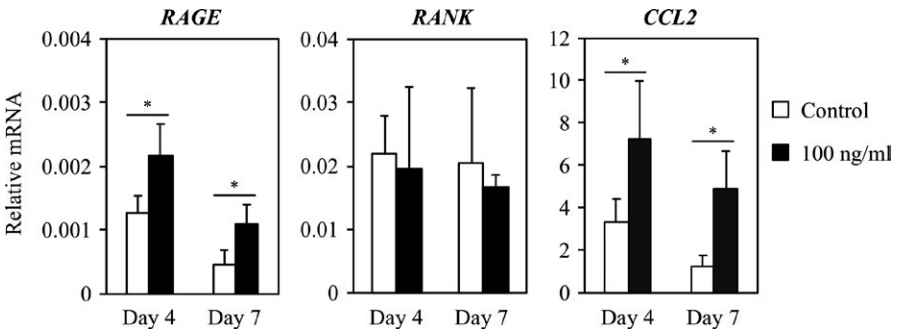

FIGURE 4 Effect of S100A7 on gene expression by osteoclasts. Human $CD14^+$ monocytes were differentiated to osteoclasts in the absence or presence of S100A7 (100 ng/ml). At day 4 and day 7 of the differentiation, the expression of *RAGE*, *RANK*, and *CCL2* was examined using by qPCR; $N = 4$. Value is expressed as mean ± *SD*. $^*p < 0.05$

1987). The odontoclast is an osteoclast-like cell located in the dental pulp and involved in the resorption of dentin (Sahara, Toyoki, Ashizawa, Deguchi, & Suzuki, 1996). The characteristics of odontoclasts are similar to those of osteoclasts; they also express cathepsin K, tartrate-resistant acid phosphatase (TRAcP), H^+-ATPase, and MMP-9 (Sahara et al., 1996).

Dental pulp cells may encounter an internal compressive stress from intrapulpal pressure, which is due to an increase in extravascular fluid caused by pulpal inflammation. Since the dental pulp tissue is surrounded by mineralized tissue, it has been reported that an inflamed dental pulp experience an almost 3 times increased intrapulpal pressure (Heyeraas & Berggreen, 1999). Orthodontic tooth movement causes tissue deformation that evokes a fluid flow, which consequently generates a shear stress to the cells (Henneman, Hoff, & Maltha, 2008). Pulpal inflammation was also observed following orthodontic tooth movement although this occurred to a lesser extent than in the periodontal ligament (Meeran, 2012; Yamaguchi & Kasai, 2007).

The in vitro mechanical compressive and shear stress model used in this study may represent stress originating from external and internal forces which may have a direct impact on inflammatory responses of HDPCs. Here, we reported that mechanical compressive force induced expression of *IL1*, *IL6*, and *VEGF* by HDPCs. Similarly, previous reports showed that compressive force upregulated *IL6* expression by stem cells isolated from human exfoliated deciduous teeth (Govitvattana et al., 2013). The production of the inflammatory mediators IL-6 and VEGF was also observed following mechanical shear force applied to osteocytes and human mesenchymal stem cells form bone marrow, respectively (Bakker, Kulkarni, Klein-Nulend, & Lems, 2014; Becquart et al., 2016). Further, cyclic mechanical strain induced the expression of various inflammatory molecules by HDPCs including *IL1*, *IL6*, and *TNF* (Lee et al., 2008). Hence, our model confirms the response of HDPCs to mechanical stimuli, suggesting a role of mechano-transduction in the regulation of dental pulp homeostasis.

Here, we demonstrated that mechanical stress upregulated the expression of S100A7 by HDPCs at both mRNA and protein level. An increased mRNA expression of S100A7 was also detected in the dental pulp tissue obtained from orthodontically moved teeth, suggesting it also plays a role in vivo. S100A7 is a relatively small calcium-binding protein, which is abundantly expressed in inflamed skin, such as psoriatic lesion, and in a tumor microenvironment (D'Amico et al., 2016; Emberley, Murphy, & Watson, 2004). Expression of *S100A7* in tissues is upregulated in response to pro-inflammatory cytokines, such as IL-1, IL-6, IL-17, and IL-22 (D'Amico et al., 2016). We also observed that HPDCs treated with IL-1 showed an increased production of S100A7 (Figure S5a). S100A7 performs important extracellular functions, and it can act as a chemokine and cytokine playing a pivotal role in the promotion and exacerbation of inflammatory responses (D'Amico et al., 2016; Jinquan et al., 1996).

Mechanical loading is sensed by the mechanosensitive P2X7 receptor (P2X7R), which is expressed in many cell types, including periodontal ligament cells and HDPCs (Kanjanamekanant, Luckprom, & Pavasant, 2013; Lu et al., 2017; Wang, Yi, Ren, & Xie, 2016). The activation of P2X7R by mechanical loading upregulated *IL1* and *IL6* expression (Kanjanamekanant et al., 2013; Lu et al., 2017), which is concordant with increased levels of *IL1* and *IL6* in mechanical stress-treated HDPCs as seen in the present study. In addition, it has been shown that the activation of P2X7R enhanced *S100A7* expression (D'Amico et al., 2016). Therefore, the increased S100A7 expression and production by HDPCs in response to mechanical loading may be via the activation of P2X7R. Further investigation is required to confirm this possibility.

S100A7 expression by breast cancer cells was implicated in bone resorption (Paruchuri et al., 2008). Further, the loss of S100A7 expression in cancer cells leads to an impaired osteoclast differentiation and a decrease in osteoclast number (Paruchuri et al., 2008). Hence, S100A7 may participate in osteoclast differentiation and function. Correspondingly, the present study demonstrated that S100A7 promoted osteoclast formation and activity.

Data presented in the literature revealed that the receptor for advanced glycation end product (RAGE) is a putative receptor of S100A7 (Wolf et al., 2008). It has been shown that $RAGE^{-/-}$ osteoclastic precursors failed to respond to *M*-CSF and RANKL (Zhou et al., 2006). RAGE is expressed by monocytes and macrophages; cells that have the capacity to differentiate into osteoclasts (Udagawa et al., 1990). The expression of RAGE is induced by RANKL and is upregulated upon osteoclast differentiation (Zhou et al., 2006). $RAGE^{-/-}$ mice exhibited an increased bone mass due to an impaired osteoclast formation and activity (Zhou et al., 2006). As mentioned above, S100A7 promoted osteoclast differentiation and activity by enhancing the effect of *M*-CSF and RANKL. In addition, the present study also demonstrated that S100A7 upregulated *RAGE* expression by osteoclasts, implying that S100A7 may promote osteoclast differentiation via enhancing the expression of this receptor. Activation of RAGE by its ligands induces various downstream signaling molecules, including NF-B which subsequently activates target genes involved in inflammatory responses by various cell types including monocytes and macrophages (Gu et al., 2006; Han, Kim, & Mook-Jung, 2011). *CCL2* is one of the well-known target genes activated by RAGE (Gu et al., 2006; Tesch, 2008). CCL2 plays a role in activation of osteoclasts and was upregulated by S100A7 (Hayakawa, Yoshimoto, Sekizawa, Sugiyama, & Hirata, 2012; Nasser et al., 2012). In this study, we observed also an upregulation of *CCL2* similar to that of *RAGE* when stimulated with S100A7 (Figure 4). These findings support the involvement of RAGE and its target genes in S100A7-enhanced osteoclastogenesis.

In conclusion, we elucidated a possible molecular mechanism of root resorption caused by mechanical stress. When the dental pulp cells experience mechanical stress, the cells secrete inflammatory mediators, among which S100A7. Subsequently, S100A7 promotes the differentiation of monocytes to osteoclasts/odontoclasts and enhances osteoclast resorption activity, thus contributing to root resorption. This study improved our understanding of the mechanisms underlying root resorption caused by mechanical stress and pulpal inflammation.

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CONFLICT OF INTEREST

All authors have none to declare.

AUTHOR CONTRIBUTION

H.C.: Performed all experiments, analyzed data, prepared figures; T.O., P.P.: Contributed human dental pulp cell isolation, and help with manuscript writing and editing; N.L., B.K., C. NL.: Generated and contributed Shear Force Loading Apparatus; V.C.: Contributed flow cytometric analyses. T.P.: supervised osteoclast differentiation and pit resorption assay. V.L.: help with manuscript writing and editing. P.R.: designed and coordinated the project, supervised all experiments, process the data, and wrote and edited the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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