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A novel *in vitro* loading system to produce supraphysiologic oscillatory fluid shear stress

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Abstract

A multi-well fluid loading (MFL) system was developed to deliver oscillatory subphysiologic to supraphysiologic fluid shear stresses to cell monolayers *in vitro* using standard multi-well culture plates. Computational fluid dynamics modeling with fluid-structure interactions was used to quantify the squeeze film fluid flow between an axially displaced piston and the well plate surface. Adjusting the cone angle of the piston base modulated the fluid pressure, velocity, and shear stress magnitudes. Modeling results showed that there was near uniform fluid shear stress across the well with a linear drop in pressure across the radius of the well. Using the MFL system, RAW 264.7 osteoclastic cells were exposed to oscillatory fluid shear stresses of 0, 0.5, 1.5, 4, 6, and 17 Pa. Cells were loaded 1 h per day at 1 Hz for two days. Compared to sub-physiologic and physiologic levels, supraphysiologic oscillatory fluid shear induced upregulation of osteoclastic activity as measured by tartrate-resistant acid phosphatase activity and formation of mineral resorption pits. Cell number remained constant across all treatment groups.

Keywords

Fluid shear stress; Osteoclast; Mechanoregulation; Supraphysiologic fluid shear stress

1. Introduction

Modulation of bone cell behavior via mechanotransduction is a significant regulator of skeletal homeostasis, with the primary cellular stimulus being local fluid shear stress generated through whole bone loading during physical activity. The ability of whole bone loading to generate squeeze flow, and thus fluid shear through osteocyte lacunae has been extensively documented (Cardoso et al., 2013; Cowin et al., 1995; Knothe Tate, 2003; Knothe Tate and Knothe, 2000; Knothe Tate et al., 1998, 2000; Kufahl and Saha, 1990; Price et al., 2010, 2011; Srinivasan and Gross, 2000; Wang et al., 2000). Using *in vitro* models, the mechanosensitivity of osteoblasts and osteoclasts to their local physiologic fluid shear stress has been documented in parallel-plate flow chamber models (Batra et al., 2005; Case et al., 2010; Jacobs et al., 1998; Kim et al., 2006; Tan et al., 2007). While the true magnitude of shear stress experienced by bone cells *in vivo* is not known, estimates typically hold “physiologic” shear stress to range between 0.5 and 1.5 Pa (Weinbaum et al., 1994). Using a mixture theory model of vertebral cancellous bone, Dickerson et al. (2008) calculated the range of interfacial shear stresses over trabeculae during *in vivo* vibrational

loading to be 0.1–2.0 Pa. Applying Biot's poroelastic media theory to models of the lacunar–canalicular porosity, Weinbaum and Cowin calculated local fluid shear to range from 0.8 to 3 Pa, with a predicted value of 1 Pa during physiologic skeletal loading (typical walking loads at 1 Hz) (Biot, 1955; Cowin et al., 1995; Weinbaum et al., 1994). Similarly, Anderson and Knothe Tate applied computational modeling to 3-D reconstructions of transmission electron micrographs of canaliculi, deriving a dimension-less maximal shear stress value of 0.77 (Anderson and Knothe Tate, 2008). Price et al. (2011) used FRAP (fluorescence recovery after photobleaching) imaging methods on sodium fluorescein-labeled mouse tibiae under cyclic compression loading to calculate a maximum fluid shear stress of 5 Pa. Similarly, Mi et al. (2005) calculated maximum fluid shear stresses in avian long bone loading to range between 0.2 Pa (rooster running) and 1 Pa (turkey ulna bending).

While physiologic skeletal loading typically produces estimated shear stresses on the order of ~ 1 Pa, there exist microenvironments in which osteoblasts and osteoclasts may be subjected to supraphysiologic shear stresses. One such microenvironment occurs at the interlocked cement–bone interface in total knee prostheses during joint loading, where fluid may be pumped between the trabeculae and cement through very thin interface gaps. In these cases, shear stresses may exceed 10 Pa at the bone surface (Mann and Miller, 2013a). Postmortem retrieved knee replacements reveal a loss of interlock between cement and trabecular bone where initially interdigitated trabeculae resorb, leaving an empty cavity in the cement (Mann et al., 2012). We postulate that supraphysiologic shear stress may directly upregulate osteoclast activity at this interface via mechanobiological mechanisms, and that this may represent a mechanism driving loss of cement–bone micro-interlock and contribute to aseptic implant loosening.

The mechanosensitivity of osteoclastic cells has been demonstrated *in vitro* using Flexcell, hydrostatic pressure, and fluid flow systems (Ferrier et al., 2000; Guo et al., 2010; Kim et al., 2006; McAllister et al., 2000; McEvoy et al., 2002; Suzuki et al., 2008). Using a parallel plate flow chamber model, McAllister et al. found that fluid shear stress (1.6 Pa for 6 h) increased nitric oxide and prostaglandin E_2 production in primary rat pre-osteoclasts (McAllister et al., 2000). Exposing primary rat pre-osteoclasts to short durations (30 min) of fluid shear (0.09–2.63 Pa), Guo et al. noted early increases in osteoclast cell area and expression of vacuolar H^+ -ATPase subunit genes (ATP6V1A and TCIRG1) (Guo et al., 2010). Using a co-culture of ST-2 murine bone marrow stromal cells with RAW 264.7 osteoclastic cells, Kim et al. (2006) demonstrated time-dependent increases in the RANKL/OPG mRNA ratio and decreases in tartrate-resistant acid phosphatase staining following one hour of oscillatory fluid flow exposure in a parallel plate flow chamber (1 Pa peak shear stress at 1 Hz). Collectively, these studies demonstrate the ability of “physiologic” fluid shear stress to modulate osteoclast activity *in vitro*. To our knowledge, however, no *in vitro* model has investigated the effects of supraphysiologic fluid shear stresses on osteoclasts.

The goals of the present study were to (1) develop a novel *multi-well fluid loading* (MFL) system capable of producing oscillatory subphysiologic to supraphysiologic fluid shear stresses to cell monolayers *in vitro*, and (2) use this system to determine if supraphysiologic fluid shear stresses modulate osteoclast resorptive activity *in vitro*.

2. Methods

2.1. Oscillatory flow via a squeeze film phenomenon

In this newly devised system, oscillatory fluid flow (OFF) is created by the up and down excursion of a loading piston at the bottom of a cell culture well plate (Fig. 1A). Fluid is squeezed in and out of the gap between the bottom face of the piston and the well plate. The geometric parameters of the piston design (Fig. 1B) including diameter (D) and cone angle

of the piston (θ), along with gap distance (G), excursion of the piston (E), loading rate, and fluid viscosity result in a range of fluid flow responses. For this study, the gap ($G = 0.13$ mm) between piston and plate (at the extent of excursion), piston diameter ($D = 12.7$ mm), and well diameter ($W = 15.9$ mm) were held constant.

2.2. Multi-well fluid loading system

An array of 24 polycarbonate loading pistons with cone angles of 0, 1, 2, 5, and 10 degrees (4 each) was built into a CNC machined well plate cover and was fabricated from polycarbonate sheet (autoclave compatible for sterilization). The piston array plate was attached to a loading device (Fig. 1C) consisting of an electric motor with gear reducer, a cam mechanism, follower bearing, and linear slider mechanism. The cam mechanism could be adjusted to control the magnitude of the piston excursion (E). The DC voltage applied to the motor was adjusted to control the frequency of oscillatory motion. As designed, the system was operated in displacement control. The MFL system was placed in a humidified 5% CO₂ incubator for the duration of loading studies and a lead wire was fed to an external DC power supply.

Loading piston height was set using an electronic height gage (0.01 mm resolution) (Swiss Precision Instruments, Garden Grove, California). Gap size was set using micro-adjustment screws on the piston array plate using the same electronic height gage. The horizontal position of the plate relative the loading pistons was controlled by a lip on the piston array plate that captured the multi-well plate and prevented side-to-side displacement during loading. Excursion was set using an electronic drop indicator (0.01 mm resolution) (Swiss Precision Instruments, Garden Grove, California).

2.3. Computational fluid dynamics modeling

A closed form solution for fluid flow for the MFL design configuration does not exist. Therefore, a computational fluid dynamics (CFD) modeling approach with fluid structure interactions (FSI) was used to determine the fluid flow response for a range of cone angle and loading piston excursions (COMSOL, Burlington, MA). Structural modeling was included for the solid components (piston and well plate) in order to explore the possibility of FSI for cases where there might be appreciable deformation of the well plate.

Axisymmetric finite element models (Fig. 1D) were created with quadratic, linear elastic solid elements for the piston and well plate ($E = 2.5$ GPa, $\mu = 0.3$, $\rho = 1200$ kg/m³). P2–P1 elements were used for the fluid components ($\nu = 0.033$ Pa-s, $\rho = 1000$ kg/m³) with quadratic (P2) elements for the velocity field and linear (P1) elements for the pressure field. The fluid was considered to be Newtonian (see experiment below) and incompressible, with laminar flow conditions. A fully coupled arbitrary Lagrangian–Eulerian (ALE) method was used to model the interaction between solid and fluid phases. Models were analyzed using excursions of 0.25 and 0.5 mm and cone angles of 0, 1, 2, 5, and 10 degrees. Models had approximately 3500 elements and 23,000 degrees of freedom. The piston was displaced using a sinusoidal loading pattern. There was a no-slip boundary between fluid and solid.

2.4. CFD model validation

Fluids compressed between two parallel plates that are in close proximity are termed “squeeze flows” and these have been used widely in the field of rheology (Engmann et al., 2005; Phan-Thien, 1980). For cases where the gap height is small relative to platen radius, and with small platen excursions and strains, closed form solutions exist for descriptions of fluid velocity and shear rate. Results from the closed form solution were compared to the CFD models that approximate the parallel plate squeeze flow. CFD models were run with a 0 degree cone angle (flat plate), with a standard well plate/piston geometry (0.13 mm gap). Excursions (E) of 0.01, 0.1, and 0.5 mm (1.59, 15.9, and 79.4% strain) were applied at a

loading rate of 1 Hz. Fluid velocity data were collected at time = 0.25 s along the midline of the gap and compared to the closed form solution:

$$u = -\frac{3}{4} \frac{\dot{g}}{g} r$$

where g is the half gap separation ($G/2$), $\dot{g}$ is the derivative with respect to time for g , and r is the radial position (Anderson and Knothe Tate, 2008).

2.5. CFD convergence study

A convergence study was performed for a representative CFD model configuration with 2 degree cone angle and 0.5 mm of excursion. To assess the effect of element order, P1–P1 (linear elements for velocity and pressure) and P2–P1 (quadratic elements for velocity and linear elements for pressure) element formulations were used for the fluid elements. To assess the mesh density, the number of elements and degrees of freedom (dof) were increased from a very small number (~ 2000 dof) to a very large number ($\sim 100,000$ dof). Quadratic elements were used for the solid elements. Shear stress at the well plate surface 5 mm from the central axis was used as the outcome measure.

2.6. MFL sensitivity study

A sensitivity study was performed on each of the key geometric parameters (W , D , G , θ , E from Fig. 1) using the CFD models. The experimental resolution for each parameter is given in Table 1, along with the nominal variables used in the sensitivity study. Parameters D , G , θ , and E were based on resolution of the experimental digital indicators. The well plate diameter resolution was based on the total possible transverse positioning error of the well plate with respect to the loading piston. Each parameter was increased by the experimental resolution magnitude and the resulting peak fluid shear stress, velocity, and pressure were compared to the baseline configuration. Error magnitudes and percentages were calculated.

2.7. Osteoclast culture

RAW 264.7 osteoclastic cells (ATCC, Manassas, VA) were plated at a density of 10,000 cells/cm² in 24-well plates ($n = 4$ plates each for crystal violet and TRAP staining, $n = 2$ plates for dsDNA quantification). Four additional commercially available 24-well plates with ceramic-coated culture surfaces (OsteoAssay, Corning, Tewksbury, MA) plates were seeded at the same density for quantification of mineral resorption. Media was α -MEM (Mediatech, Manassas, VA) supplemented with 10% FBS (Atlanta Biologicals, Lawrenceville, GA), 1% antibiotic/antimycotic (Mediatech, Manassas, VA), and 50 ng/ml RANK-L (PeproTech, Rocky Hill, NJ) to induce osteoclastic differentiation.

On the third day of culture, media was changed and was supplemented with 10% dextran ($M_r \sim 2 \times 10^6$, Sigma-Aldrich, St. Louis, MO), raising the media viscosity to 0.033 Pa-s at 37 °C. Media viscosity was determined using a cone and plate rheometer (37 °C, 2 degree cone angle, 1°/s rotation, Rheology Advantage AR2000ex, TA Instruments, New Castle, DE). Newtonian fluid behavior was confirmed by performing a dynamic sweep of shear rates from 1 to 100 s⁻¹. Cyclic loading using the MFL piston array was done on days 3 and 4 of culture for 1 h per day at 1 Hz with an excursion of 0.5 mm. Cells were exposed to calculated shear stresses of 0.5, 1.5, 4, 6, and 17 Pa, with static (0 Pa) controls ($n = 4$ wells per shear stress per well plate). As a control, an acellular OsteoAssay plate was treated using identical media and loading conditions in order to determine if oscillatory fluid shear induced mineral resorption in the absence of osteoclastic cells.

Cell cultures were fixed on day 5 and stained with crystal violet ($n = 16$ wells per shear stress) or for tartrate-resistant acid phosphatase (TRAP) activity ($n = 16$ wells per shear stress, acid phosphatase kit 387A, Sigma-Aldrich, St. Louis, MO). OsteoAssay plates were rinsed with 10% bleach for 5 min and allowed to dry ($n = 16$ wells per shear stress).

Wells were then imaged and analyzed for stained or resorbed area, staining intensity, and grayscale intensity (total mineral resorbed) using NIH ImageJ. ImageJ analysis was restricted to the region directly under the loading piston, excluding the area between the outer edge of the piston and well sidewall. Using the OsteoAssay plates, regional analysis of the resorption pit area as a function of distance from the center of the well was conducted. Resorbed area fraction was calculated using concentric rings originating at the center of the well and advancing radially in increments of 1 mm. DNA content per well was quantified using two representative 24-well plates ($n = 8$ wells per shear stress) using the AccuBlue dsDNA quantitation kit (Biotium, Hayward, CA).

2.8. Statistical analysis

Statistical analysis included analysis of variance and pairwise comparisons (Tukey-Kramer) to determine the effects of fluid shear stress on TRAP+ area, TRAP staining intensity, and total resorbed mineral area (JMP 10, SAS, Cary, NC). Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Multi-well fluid loading system

Oscillatory loading caused a reversal of flow direction during the compression phase (0 to 0.5 s) and extension phase (0.5 to 1 s) of the piston loading cycle (Fig. 2). The maximum velocity was near the outer edge of the piston and occurred at ~ 0.35 s and ~ 0.7 s with 1 Hz loading. Fluid shear stress on the well plate (Fig. 3A) was nearly uniform over the majority of plate surface. There were low shear stress regions beyond the edge and at the center of the piston axis. Maximum shear stress also occurred twice per cycle at approximately 0.35 and 0.7 s. Fluid pressure (Fig. 3B) was highest near the tip of the cone (88 Pa for a 2 degree cone angle with 0.25 mm excursion). There was a linear drop in fluid pressure moving from the cone tip to the edge of the loading piston.

Increasing the cone angle from 0 to 10 degrees resulted in decreasing maximum fluid pressure, shear rate, and velocity (Fig. 4). This small change in cone angle resulted in a near two orders of magnitude change in pressure and shear stress, and a one order of magnitude change in fluid velocity. Increasing the piston excursion caused a shift in the flow-cone angle response curves. The response was not proportional though; doubling excursion did not double pressure, shear rate, or fluid velocity.

The validation study showed that for small oscillatory strains ($E = 0.01$ mm, $\varepsilon < 2\%$), the ratio of fluid velocity for the CFD and closed form solution was 1.0, indicating that there was a good match between the CFD results the closed form solution (Fig. 5). Towards the edge (~ 6 mm) of the piston the CFD fluid velocities were slightly lower than the closed form solution due to the presence of the fillet. As the applied excursions increase in magnitude, the small strain assumption no longer applied and the discrepancy between CFD and closed form solution increased.

For the convergence study, there was an increase in shear stress for the P1-P1 element formulation with total element degrees of freedom (dof). Even with 100,000 dof, it does not appear that the shear stress had completely converged for the P1-P1 formulation (Fig. 6). For the P2-P1 formulation, a constant (3.85 Pa) shear stress was reached using 13,000 dof;

further increases in dof did not change shear stress. The convergence study shows that the chosen element type (P2-P1) and resolution ($\sim 23,000$ dof) was appropriate to reach convergence for the models.

The sensitivity analysis (Table 1) showed that the piston diameter (D) and well plate diameter (W) parameters were the least sensitive to uncertainty in experimental measurement. The gap distance (G) and excursion (E) were the most sensitive. Maximum errors were 0.25 Pa (6.5%) for fluid shear stress, 0.4 mm/s, (3.1%) for fluid velocity, and 18 Pa (13.8%) for fluid pressure.

3.2. Osteoclast loading

Osteoclast activity, as quantified by TRAP staining and mineral resorption, was modulated by shear stress (Fig. 7). No loss of mineral was detected in the acellular OsteoAssay plate, indicating that fluid shear alone did not induce mineral resorption. Shear stress was a significant predictor of both total TRAP activity ($p < 0.0001$) and mineral resorption ($p = 0.0175$). Supraphysiologic shear stress significantly elevated TRAP activity compared to both static (0 Pa, $p < 0.0001$) and physiologic shear stresses (0.5 and 1.5 Pa, $p < 0.0001$). Exposure to a shear stress of 0.5 Pa also decreased mineral resorption compared to supraphysiologic shear stresses (4, 6, and 17 Pa, Fig. 8). Total resorbed area, TRAP+ area, and cellularized (crystal violet stained) areas were not affected by shear stress, indicating a modulation of osteoclastic activity rather than cell number. Likewise, the dsDNA content remained equivalent across treatment groups. Spatial distribution analysis of resorption pit formation indicated a consistent decrease in resorbed mineral fraction with increasing distance from the center of the well. This result was consistent across all treatment groups, including non-loaded controls. Spatial distribution of resorbed area fraction was significantly different only between the 0.5 and 17 Pa treatment groups ($p = 0.0065$).

4. Discussion

A computational fluid dynamics modeling approach was used to guide the design of the MFL system. The cone angles in the experimental setup (0, 1, 2, 5 and 10 degree) were chosen to span a wide range of fluid shear stress responses from sub- to supraphysiologic (Jacobs et al., 1998; Mann and Miller, 2013a, 2013b). Additionally, the MFL system was adapted for use with standard multi-well cell culture plates, and designed to load multiple samples in replicate within the multi-well plate. This system permits higher sample throughput than PPF systems do, as replicates can be run within the same well plate. The MFL system is also capable of producing a wide range of fluid shear stresses with only minor hardware modifications, enabling us to screen a wide range of applied shear stresses within each experiment.

Osteoclasts cultured in this system demonstrate shear stress-dependent responses in cell activity, indicating that (1) our loading system is capable of inducing differential responses in cultured cells, and (2) osteoclast activity is upregulated by supraphysiologic fluid shear stress. Specifically, we noted supraphysiologic shear stress induced increased production of tartrate-resistant acid phosphatase, which is required for resorption of bone mineral *in vivo*, and increased mineral resorption. Cell number was unaffected by shear stress, indicating that supraphysiologic shear stress induced increased osteoclast resorptive activity, rather than modulating cell proliferation or viability. Our outcome measures focused on changes in osteoclast functional behavior (mineral resorption and TRAP production) rather than alterations in gene expression. This is in contrast to the few published studies of osteoclast mechano-activation, which have focused primarily on short-term changes in gene expression and nitric oxide production (Guo et al., 2010; Kim et al., 2006; McAllister et al., 2000; McEvoy et al., 2002; Suzuki et al., 2008). While alterations in gene expression are of

interest in identifying pathways leading to osteoclast activation, the objective of this study was ultimately to demonstrate the ability of supraphysiologic shear stress to enhance osteoclastic mineral resorption.

The limitations of the MFL system are common to the PPF systems as well. First, the shear stress distribution is not completely uniform over the entire well plate surface. Parallel-plate flow (PPF) chambers are also subject to non-uniform shear stress in regions where flow profiles are not fully developed (fluid inlet and outlet). Second, the loading pistons require precise spacing in relation to the well plate surface because the fluid response is sensitive to the gap distance. Third, the pressures required to drive the fluid from the gap between the piston and the well plate (maximum of 722 Pa) are not constant over the well plate surface. However, this variation in pressure is also experienced in parallel plate flow chambers. To characterize differences between the MFL system and the fluid response for a PPF system with oscillatory fluid flow, a computational fluid dynamics model was created for a PPF system (Jacobs et al., 1998). For a gap of 0.28 mm and plate length of 60 mm, a peak fluid pressure of 7730 Pa is required with the PPF system to achieve the peak fluid shear stress used in this study (17 Pa)—an order of magnitude higher than in the MFL system. Higher pressures are required in the PPF system as the length over which the fluid is pumped (60 mm) is an order of magnitude larger than the length over which the fluid is pumped (piston radius = 6.3 mm) in the MFL design. To put this in context, McEvoy et al. (2002) found that primary macrophages could be activated to express pro-osteoclastic genes using cyclic hydrostatic loading only when pressures reached 35,000 Pa. Similarly, Ferrier et al. (2000) required cyclic hydrostatic pressures of 138,000 Pa to induce upregulation of interleukin-1 β and tumor necrosis- α gene expression in primary human monocytes. It is unlikely that the fluid pressures generated in the MFL system contribute to modulation of osteoclast activity.

Our finding of increased osteoclastic activity in response to supraphysiologic shear stress aligns with previous observations from *in vivo* studies. Using an *in vivo* rat model of localized bone loading, Fahlgren et al. determined that cortical surface pressures of $\sim$ 52,000 Pa induce extensive bone resorption, while a physiologic intramedullary pressure load of 8000 Pa induces an anabolic response (Fahlgren et al., 2010; Qin et al., 2003). In their model the brief, extreme spikes in fluid pressure produced by actuating a transcutaneous piston translate to short bursts of high-velocity ($\sim$ 20 mm/s) fluid flow leading to localized osteolysis (Fahlgren et al., 2010). While it is not possible to directly compare our *in vitro* results with existing *in vivo* data, our results in combination with Fahlgren's data provide evidence that supraphysiologic fluid flow may directly contribute to aseptic osteolysis. Although there are multiple mechanisms *in vivo* that may contribute to fluid flow-induced osteolysis, our data also support the theory that increased osteoclastic activity may be a significant contributing factor. These data illustrate the ability of supraphysiologic fluid flow conditions to modulate bone homeostasis in a highly localized manner analogous to conditions present at the implant-bone interface.

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Fig.1.

(A) Cyclic excursion of a loading piston causes fluid to be pumped in and out of the gap between the piston base and well plate. (B) Adjusting parameters such as the minimum gap distance (G), cone angle (θ), and loading rate affects the fluid flow response on the well plate surface. (C) The system was designed to accommodate a range of loading piston load angles using a piston array plate attached to the MFL system. (D) Fluid flow response was simulated using the finite element method.

Fig. 2. Fluid velocity sequence for a 1 Hz loading cycle illustrates reversing flow direction as piston moves down and then up.

Fig. 3. Distribution of (A) fluid shear stress and (B) pressure on the well plate surface for an excursion of 0.25 mm, cone angle of 2 degrees, and 0.033 Pa-s viscosity.

Fig. 4. Maximum velocity, shear stress and pressure were determined as a function of piston cone angle and excursion (E) magnitude for a 1 Hz loading rate with fluid viscosity of 0.033 Pa-s.

Fig. 5.

Computational fluid dynamics solution (CFD) was compared to the closed form solution for excursion (E) magnitudes of 0.01, 0.1, and 0.5 mm for the case with 0 degree cone angle. The fluid velocity ratio (CFD/closed form solution) was near 1.0 for small excursions, but deviated for large excursions.

Fig. 6.

Fluid shear stress as a function of mesh degrees of freedom for a well plate with 2 degree piston angle and 0.5 mm of excursion. Shear stress along the well plate surface 5 mm from the central axis of the piston is shown for results with P1-P1 and P2-P1 fluid element formulations.

Fig. 7. Resorption pits in mineralized substrate formed by osteoclasts under (A) supraphysiologic fluid shear stress (17 Pa), (B) static (0 Pa), and (C) lack of mineral resorption under acellular conditions (17 Pa). The white circle denotes loading piston cone zone.

Fig. 8. Fold changes in (A) total resorbed area, (B) TRAP+ area, and (C) TRAP staining intensity as a function of fluid shear stress.

Table 1

Sensitivity analysis for geometric parameters used in the multiwell plate loading system. Well plate diameter (D), piston-plate gap distance (G), cone angle (θ), and excursion (E) were adjusted from baseline values.

Parameter	Baseline value	Experimental resolution	Error magnitude		Error (%)			
			Shear Stress (Pa)	Velocity (mm/s)	Pressure (P)	Shear stress (Pa)	Velocity (mm/s)	Pressure (P)
W (mm)	15.87	0.25	-0.002	0.000	-0.330	0.06	0.00	0.25
D (mm)	12.7	0.02	-0.001	-0.100	0.220	0.03	0.76	0.17
G (mm)	0.63	0.01	-0.198	-0.300	-13.86	5.13	2.29	10.45
Θ (degree)	2	0.02	-0.039	-0.100	-1.50	1.01	0.76	1.13
E (mm)	0.5	0.01	0.250	0.400	18.32	6.5	3.05	13.82