

Multiphysics simulation of a compression - perfusion combined bioreactor to predict the mechanical microenvironment during bone metastatic breast cancer loading experiments

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Abstract

Incurable breast cancer bone metastasis causes widespread bone loss, resulting in fragility, pain, increased fracture risk, and ultimately increased patient mortality. Increased mechanical signals in the skeleton are anabolic and protect against bone loss, and they may also do so during osteolytic bone metastasis. Skeletal mechanical signals include interdependent tissue deformations and interstitial fluid flow, but how metastatic tumor cells respond to each of these individual signals remains under-investigated, a barrier to translation to the clinic. To delineate their respective roles, we report computed estimates of the internal mechanical field of a bone-mimetic scaffold undergoing combinations of high and low compression and perfusion using multiphysics simulations. Simulations were conducted in advance of multi-modal loading bioreactor experiments with bone metastatic breast cancer cells to ensure that mechanical stimuli occurring internally were physiological and anabolic. Our results show that mechanical stimuli throughout the scaffold were within the anabolic range of bone cells in all loading configurations, were homogeneously distributed throughout, and that combined high magnitude compression and perfusion synergized to produce the largest wall shear stresses within the scaffold. These simulations, when combined with experiments, will shed light on how increased mechanical loading in the skeleton may confer anti-tumorigenic effects during metastasis.

Introduction

Roughly three in four patients with advanced breast cancer develop incurable bone metastases (Siegel, Miller, & Jemal, 2018). Once bone metastasis occurs, the lesions are overwhelmingly osteolytic, putting patients at great risk of suffering skeletal related events (SREs), including severe bone pain and fracture (Coleman, 2005). The mechanical environment in the skeleton is well-known to control bone tissue homeostasis, with increased loading preventing or reversing osteoporotic bone loss and associated fracture risk (von Stengel, Kemmler, Kalender, Engelke, & Lauber, 2007). Similarly, mechanical signals are emerging as a critical factor in bone metastasis and tumor-induced bone disease (TIBD) (Beaton et al., 2009; Sheill, Guinan, Peat, & Hussey, 2018). A few promising reports showed that in patients with existing bone metastases, physical therapy resulted in multiple positive physical outcomes (*e.g.* increased muscle strength) without SREs (Cormie et al., 2013; Galvao et al., 2018), although changes to bone mass and architecture were not explicitly evaluated. In preclinical models, increased loading was protective against TIBD, such as tibial compression (Fan et al., 2020; Lynch et al., 2013), low intensity vibrations (Pagnotti et al., 2016), and ankle loading (Yang et al., 2019). But, the impacts of loading on tumor cell function may be dose-dependent, where high damage-causing forces may reverse the protective effects of loading against TIBD (Fan et al., 2020). Clearly, more work is needed to clarify the impacts of anabolic loading on TIBD before successful translation to the clinic.

When forces are applied to the skeleton during physical activity, the deforming tissue pressurizes the interstitial fluid, resulting in fluid flow from high to low pressure. These interdependent physical signals are then translated into intracellular biochemical signals, thereby stimulating bone formation when increased (Robling & Turner, 2009; Thompson, Rubin, & Rubin, 2012). Thus, elucidating the mechanisms of how each of these mechanical signals is translated into stimuli in cancer cells within a bone microenvironment is needed to understand how anabolic loading is anti-tumorigenic. To this end, tissue engineering-based approaches have been an indispensable tool. Loading-induced interstitial fluid flow is widely recognized as an anabolic signal for stimulating osteogenesis and bone formation (McCoy & O'Brien, 2010), and can be generated in scaffolds via directly-applied perfusion or compression of a hydrated scaffold. Recently, these approaches have been leveraged to help uncover how skeletal mechanical signals impact cancer cells in bone. Dynamic compression applied to breast cancer cells in a mineral-containing polymeric scaffold downregulated expression of osteolytic genes (Lynch et al., 2013). In contrast, dynamic compression applied to Ewing Sarcoma cells in a hydrogel increased their drug resistance (Marturano-Kruik et al., 2018; Santoro, Lamhamedi-Cherradi, Menegaz, Ludwig, & Mikos, 2015), underscoring that cancer type and microenvironment are important factors in bone cancer mechanobiology. Mechanical signals also regulate tumor cell interactions with resident bone cells. For example, mesenchymal stem cells (MSCs) increased their osteopontin production upon exposure to breast cancer-derived soluble factors during compression-induced osteogenic differentiation (Lynch et al., 2016), indicating that tumor cells may stimulate bone cells to secrete proteins that promote tumor cell adhesion. Further, mechanically loaded, paclitaxel-releasing MSCs inhibited the growth of multiple myeloma cells in the Rotary Cell Culture System (Bonomi et al., 2017; Ferrarini et al., 2013).

While fluid flow is clearly important for bone homeostasis, the impact of matrix deformations is much less understood. Matrix deformations and fluid flow occur together (Robling & Turner, 2009), thus delineating the individual roles of each signal in cancer cell mechanobiology studies is challenging. One approach is to apply perfusion and compression in combination and isolation, and several recent studies using this approach report that fluid flow- and compression-induced signals together enhance bone anabolism (Ramani-Mohan et al., 2018; Zhao, Mc Garrigle, Vaughan, & McNamara, 2018; Zhao, Vaughan, & McNamara, 2015). For example, multiscale computational modeling of a hydrogel scaffold undergoing perfusion, compression, or both predicted that the combination of low magnitude (0.5% peak strain) compression and pore pressure (10 kPa) would induce more osteogenic differentiation and bone mass (Zhao et al., 2018), perhaps as a result of greater cell deformation (Zhao et al., 2015). Further, when computational approaches were combined with combinatorial experiments involving MSCs with an AP-1 (an intracellular strain sensor) luciferase reporter, applied compression resulted in the greatest cellular deformation and osteogenesis, suggesting physical strain is the main driver of bone anabolism rather than fluid flow alone (Ramani-Mohan et al., 2018). Thus, both mechanical signals should be considered when investigating the role of anabolic loading on tumor cells in the skeleton. Further, computational simulations may help shed light on their respective roles.

Here, we report on multiphysics computational simulations of multi-modal loading bioreactor experiments in advance of the metastatic breast cancer cellular experiments to ensure a mechanical environment in the anabolic range. Our bioreactor delivers compression and perfusion individually or in combination to multiple bone-mimetic 3D constructs (Fig. 1A). We have previously determined the necessary inlet flow velocity to engender physiological and anabolic wall shear stresses (WSSs) in our bone-mimetic scaffold (Liu, Han, Hedrick, Modarres-Sadeghi, & Lynch, 2018), and we also determined an osteogenic level of dynamic compression that also impacted bone cell-tumor cell interactions (Lynch et al., 2016). Here, we extend our previous work to include simulations of multiple magnitudes as well as dynamic compression, and we anticipate that the combination of applied compression and perfusion will synergize to produce the greatest mechanical signals (Zhao et al., 2018).

Materials and Methods

Overview of Experimental Setup used for Simulations

Our experimental setup includes a multi-modal loading bioreactor and bone-mimetic scaffold. With our bioreactor (Bangalore integrated System Solutions), we can apply compression and perfusion, which are

independently controlled, either in isolation or in combination. Mechanical stimuli is applied to breast cancer cells seeded in highly porous scaffolds fabricated from poly(lactide-co-glycolide) (PLGA) microspheres and hydroxyapatite (HA) scaffolds (Lynch et al., 2013; Pathi, Kowalczewski, Tadipatri, & Fischbach, 2010). We simulated eight experiments with dynamic compression and steady perfusion in various combinations with a static, nonloaded control (Fig. 1B). The compression conditions included no compression (C-), low compression (5% peak bulk strain, C+), and high compression (10% peak bulk strain, C++) applied at 1 Hz. The high compression value (10% peak strain) previously inhibited expression of resorption genes in breast cancer cells within the same PLGA-HA scaffold modeled here (Lynch et al., 2013), and modulated their interactions with bone marrow mesenchymal stems during osteogenic differentiation (Lynch et al., 2016). The low compression value (5% peak strain) serves as a lower stimulus, but is within the range of commonly used strains in experiments to stimulate osteogenic responses (Bhatt et al., 2007).

The inlet perfusion velocity conditions included no perfusion (P-), steady low perfusion (0.3 mL/min, P+), and steady high perfusion (0.6 mL/min, P++). Our rationale for applying steady, as opposed to dynamic, perfusion is that no clear consensus currently exists as to which profile is better for osteoinductive responses in bone cells in tissue engineered constructs (McCoy & O'Brien, 2010). The low perfusion rate (0.3 mL/min) was previously simulated via CFD, and resulted in internal shear stresses that are in the osteogenic range and flow velocities similar to intracanalicular flow velocities during in vivo tibial loading (Liu et al., 2018).

Geometry Reconstruction from MicroCT Images

Previously acquired micro-computed tomographic (microCT) images of the scaffold (Liu et al., 2018) (Fig. 2A) were used to generate computational models (Fig. 2B). Image data were imported into Simpleware ScanIP (Synopsys, Inc.) for segmentation and directly transformed into a volumetric mesh containing both solid and fluid domains. Images were filtered to remove noise and smoothed by applying a mean filter (filter radius = 1 pixel). Grayscale stacked images were then segmented to separate solid from the air (threshold value taken from our previous model (Liu et al., 2018)). The scaffold image data were presented as a voxel model after the segmentation. Next, a voxel model of a cylinder was created to trim the scaffold into a smaller domain (1.2 mm thickness, 2 mm diameter) for shorter computation. Boolean operation intersection between the cylinder and scaffold voxel model was used to create the final solid domain (Foley, 1996). Boolean operation subtraction was used to generate the fluid domain from the image (scaffold) voids (Fig. 2C). Finally, the two domains were meshed together by replacing existing voxels directly to volumetric tetrahedral elements. The final mesh contains both solid and fluid domain with watertight assembly and interfaces with shared nodes (Fig. 3A).

Multiphysics Computational Model Creation

Eight experimental treatments were simulated (static control omitted). The 2 perfusion alone cases (C-P+ and C-P++) were simulated using only Fluent (Workbench 19.0, ANSYS, Inc.), a CFD solver. The remaining six cases were solved using a multiphysics solver, which utilized Fluent, Transient Structural (FE solver), and System Coupling [Fluid-Structure Interface (FSI)] (Workbench 19.0, ANSYS, Inc.). To establish the CFD and multiphysics models, the solid domain was imported to Transient Structural, where the equations of motion was solved in matrix form:

$$[M] \{\ddot{x}\} + [C] \{\dot{x}\} + [K] \{x\} = \{F(t)\}$$

where $[M] \{\ddot{x}\}$ represents the inertia forces, $[C] \{\dot{x}\}$ represents the damping forces, $[K] \{x\}$ represents elastic forces, and $\{F(t)\}$ is the dynamic load vector. The material of the solid domain, which is a mixture of PLGA polymer and HA, was assumed to be isotropic elastic. A Young's modulus and Poisson's ratio of 2 GPa and 0.3, respectively, were taken from similar PLGA-based scaffolds (Agrawal & Ray, 2001; Holland, Jolly, Yasin, & Tighe, 1987; Zhao et al., 2015). The material density, 2100 kg/m³, was calculated from PLGA (Blasi, D'Souza, Selmin, & DeLuca, 2005) and HA (Larrañaga, Richard J. Lewis, & Lewis, 2007) with 1:1 weight ratio according to the fabrication protocol (Pathi et al., 2010). To simulate the dynamic compression applied to the top of the scaffold (Fig. 1A), a transient 60 μm displacement (5% bulk strain, C+) or 120 μm displacement (10% bulk strain, C++) was applied at the top surface of the solid domain with a 1 Hz

sine wave. Bottom surfaces were modeled as fixed supports. The side walls were constrained to allow only in-plane displacements. Finally, fluid-solid shared interfaces were set to allow data transfer (Fig. 3B).

The fluid domain, which filled with Dulbecco's Modified Eagle Medium, was assumed to be an incompressible fluid with a constant density (1000 kg/m^3) and dynamic viscosity ($1.45 \times 10^{-3} \text{ Pa}\cdot\text{s}$) (Olivares, Marsal, Planell, & Lacroix, 2009). In Fluent, Navier-Stokes equations written as a continuity equation:

$$\nabla \cdot (\rho u) = 0$$

and a conservation of momentum equation:

$$\frac{\partial u}{\partial t} + (u \cdot \nabla) u - \nu \nabla^2 u = -\nabla \left(\frac{p}{\rho} \right)$$

were solved, where ρ is the flow density, u is the flow velocity, p is the static pressure, and ν is the dynamic viscosity. The Pressure-Based Coupled Algorithm of Fluent was used with a convergence criterion of 10^{-3} for the velocity residual in each direction and for the continuity residual.

In each CFD model, the lateral side boundary was set as a no-slip wall. Boundary conditions of $100 \text{ }\mu\text{m/sec}$ velocity and 0 Pa pressure were defined at the bottom inlet and top outlet, respectively, to simulate the direct perfusion in conditions with low perfusion (0.3 mL/min , P+). Similarly, $200 \text{ }\mu\text{m/sec}$ bottom inlet velocity was applied to models with high perfusion (0.6 mL/min , P++). To simulate fluid flow induced by compression (C+P-, C++P-), a separate boundary condition in the CFD models was utilized in which both inlet and outlet were instead set at 0 Pa pressure. In order to couple CFD with deforming solid domains, CFD models simulating treatments with compression were set to have deformable mesh with a remesh algorithm. Finally, fluid-solid interfaces were constant (Fig. 3C).

For the multiphysics models, the FE and CFD solvers were linked together by System Coupling in order to perform FSI simulations. During the simulation, the 1 Hz loading cycle was broken into time steps while the equations in either the FE or CFD solver were solved following a staggered iteration strategy. In each time step, the fluid mesh updated its shape following solid deformation, and subsequently, the CFD simulation was solved using the new mesh. Simultaneously, the remesh algorithm was deployed when necessary to avoid highly skewed elements. Then, Transient Structural solved the FE simulation considering the applied boundary and the fluid forces that were transferred from the fluid-solid interface based on the results from the previous CFD calculation. All simulations in this study were performed on remote Linux clusters [The Massachusetts Green High Performance Computing Center (MGHPCC)] with 16 cores [Intel(R) Xeon(R) CPU E5-2650 v3 @ 2.30 GHz with 128 GB RAM]. After simulations, FE and CFD solvers were linked to PostCFD (Workbench 15.0, ANSYS) for results exporting and visualization, where velocity vectors, WSS color maps, and solid strain color maps were created.

Model Sensitivity Tests

Multiple simulations were carried out for testing model sensitivities. A mesh sensitivity test was performed to determine the appropriate element size. A total of four different models were generated in which the fluid domains were simulated in CFD using the P+ loading condition (low perfusion, $100 \text{ }\mu\text{m/sec}$ inlet velocity). The models had an element number range from $\sim 44,000$ to $\sim 504,000$, with the highest element number model was four times smaller in element sizes (both maximum and minimum element lengths) than the lowest. The resulting WSS distributions of each model were nearly identical (Suppl. Fig. 1A), indicating that our starting sensitivity model had sufficiently fine element sizes, similar to other models of tissue engineered scaffold (Zhao, Melke, Ito, van Rietbergen, & Hofmann, 2019). In fact, coarser models did not pass mesh quality tests or had internal errors. We selected the mesh with the same element sizes as our previous CFD study (minimum element length of 0.02 mm , roughly four times of initial micro-CT voxel size) to maintain consistency (Liu et al., 2018).

A second sensitivity test was performed to determine time step length used in multiphysics models. The C+P+ loading condition was applied in three different simulations with 40, 100 and 200 time steps per loading cycle (1 cycle, 1 second). Distributions of fluid-solid interface WSS of each model at 0.25 s were virtually identical (Suppl. Fig. 1B). Thus, we chose 100 time steps per loading cycle to achieve a balance between data points and simulating time. Next, we assumed the scaffold was sufficiently stiff to prevent the fluid from causing reciprocal solid deformations. To verify this, we conducted two-way FSI coupled models to compare the solid strain distributions to the compression-alone FE simulation. Identical solid strain distributions verified that fluid forces did not deform solid structures (Suppl. Fig. 1C). Thus, we utilized one-way coupling simulations, allowing only solid deformation transfers. Finally, to determine the number of loading cycles to simulate, the C+P+ loading condition was used to simulate two full loading cycles (2 sec). WSSs were calculated from the fluid-solid interface on each loading cycle, and median values of these WSS showed identical curves between the first and the second cycle (Suppl. Fig. 1D). Thus, 1 second of loading was simulated.

Results

Local scaffold strains were evenly dispersed throughout the scaffold and doubled in intensity from 5% to 10% peak strain.

As discussed previously, scaffold deformation was independent of fluid flow (i.e. the fluid did not ‘push’ back). Thus, the solid deformations resulting from applied compression in six configurations (C+P-, C+P+, C+P++, C++P-, C++P+, C++P++) of the total eight conditions could be represented by the 2 compression magnitude groups alone (C+ and C++). The maximum deformations of the 1 Hz loading cycle occurred at 0.5 sec, the time of peak compressive strain, as we expected. At this moment, heat maps on the fluid-solid interface revealed evenly distributed principal strains throughout the entire scaffold (Suppl. Fig. 2, Fig. 4A) with few sites of strain concentrations. Distributions of principal strains in C+ showed that the majority of strains (>70%) were within 5,000-20,000 $\mu\epsilon$. In C++, strain values were twice as much with 10% bulk strain compared to 5% (mean values 13,300 $\mu\epsilon$ in C+, 265,000 $\mu\epsilon$ in C++), indicating that the scaffold deformed linearly despite the complexity of the geometry (Fig. 4B).

Applied compression resulted in interstitial fluid flow with and without applied perfusion.

The resulting WSSs of each model were calculated at all saved time steps (20 in 1s) from fluid-solid interfaces. The median values of WSSs showed dynamic waveforms in all six multiphysics models during the full cycle length (Fig. 5). Bulk compressive strains of 5% and 10% resulted in substantial interstitial fluid flow that generated dynamic WSS throughout the scaffold even in the absence of applied perfusion, as shown in other FSI models of tissue engineered scaffolds (Marturano-Kruik et al., 2018; Zhao et al., 2015). Heat maps of the scaffold interior showed uniformly dispersed WSS throughout the scaffold at peak point (0.25s, Fig. 5), similar to our previous results simulating perfusion alone in irregular scaffolds (Liu et al., 2018) and that of others (Maes et al., 2012; Maes, Van Ransbeeck, Van Oosterwyck, & Verdonck, 2009). In addition, both histograms (Suppl. Fig. 3) and heat maps (Fig. 5) revealed that the majority of WSS values (1-100 mPa) were within the physiological range (Maes et al., 2012; McCoy & O’Brien, 2010; Sladkova & de Peppo, 2014).

Co-existence of applied compression and perfusion created asymmetry in WSS waveforms.

WSSs induced by applied compression alone (C+P-, C++P-) revealed symmetrical waveforms, in which no WSS was observed at 0, 0.5, and 1 seconds (see Supplemental Video 1 for WSSs of C+P- in whole loading cycles). In compression only configurations, peak WSS values were found at 0.25 sec and 0.75 sec when the moving solid boundary was at maximum velocity, according to our loading sine waveform. The peak values of median WSS were 50 mPa and 100 mPa for C+P- and C++P- configurations, respectively. In contrast, when compression and perfusion were combined, WSS waveforms showed temporal asymmetry in the resulting median WSS. Peak WSSs were higher in the first half of the loading cycle (see Supplemental Video 2&3 for WSSs of C+P+ and C+P++ in whole loading cycles), suggesting that compression-induced fluid flow ‘cancels’ applied perfusion in the second half of the loading cycle. To investigate interstitial flow more deeply, we selected C+P- and C+P+ simulations to generate fluid velocity vectors and WSS heat

maps at 0.25, 0.5, and 0.75 sec (Fig. 6). At 0.25 sec, the scaffold walls had maximum downward velocity (~ 200 $\mu\text{m}/\text{sec}$ at the top boundary), while at 0.75 sec, the walls had maximum upward velocity. In the compression only (C+P-) simulation, compression pushed the fluid out with the moving scaffold boundary at 0.25 sec, which was then resorbed at 0.75 sec with a similar velocity profile as compression was unloaded (Fig. 6A). Hence the double peak symmetrical WSS waveform. However, in C+P+ simulation, the moving scaffold walls opposed the applied steady fluid flow for the first 0.5 sec. As the fluid was incompressible, the opposing velocity augmented the total upward fluid flow in the scaffold, thus generated much higher WSSs (Fig. 6B). In contrast, at 0.75 sec, the scaffold walls synergized with the direction of applied fluid flow in the upper portion of the scaffold, which reduced the total velocity and WSSs, and even created a downward flow at some local sites.

Discussion

Here, we report estimates of the internal mechanical field from multiphysics simulations of a bone scaffold undergoing combinations of high and low compression and perfusion. Simulations were conducted in advance of multi-modal experiments with bone metastatic breast cancer cells to ensure that mechanical stimuli occurring internally were anabolic. Our results show that mechanical stimuli throughout the scaffold were within the anabolic range of bone cells in all loading configurations, and local distributions were homogeneously distributed throughout.

Overall, the wall shear stresses within the scaffold during loading were found to be in the physiological and anabolic range. We reported peak median wall shear stresses in the range of $\sim 5 - 25$ mPa across all of our loading configurations, which is in line with values reported to stimulate osteogenesis in tissue-engineered scaffolds (Fernandez-Yague et al., 2015; Stops, Heraty, Browne, O'Brien, & McHugh, 2010). Some studies reported significant osteoblastic cell loss at higher WSS ranges ($\sim 1 - 50$ mPa) (Jaasma & O'Brien, 2008; Partap, Plunkett, Kelly, & O'Brien, 2010; Plunkett, Partap, & O'Brien, 2010), though how breast cancer cells will adhere to our bone scaffold at the higher WSS values is unknown. In vivo, breast cancer cells have been shown to localize to osteogenic niches at the endosteal surface (Wang et al., 2015), a site that can experience very high shear stresses according to simulations, particularly under applied loading (as high as 5 Pa) (Birmingham et al., 2015; Coughlin & Niebur, 2012). Even so, at our higher magnitude loading configurations, we and others should take care to investigate this. When breast cancer cells were seeded in the same scaffold modeled here and underwent 10% dynamic compression, the breast cancer cells' expression of genes that controlled downstream remodeling (Runx2) was altered with no apparent loss of cellularity (based on imaging) (Lynch et al., 2013). Here, our modeling results of this experiment would indicate that these cells would have experienced peak median WSS of ~ 10 mPa. Ewing Sarcoma cells in a compressed hydrogel experienced peak fluid velocities $\sim 4 - 6$ $\mu\text{m}/\text{sec}$ (Marturano-Kruik et al., 2018), which is 3 orders of magnitude different compared to our peak of 2 mm/sec. This highlights that differences in tumor cell response may occur across different cancer types and microenvironments.

Though our WSS values are in the range for osteogenesis and bone formation by osteoblastic cells, we utilized steady flow, which is not physiological. However, in vitro, whether steady or dynamic flow is more beneficial remains an open question. In 2D studies that directly compared steady versus dynamic flow, no differences were observed (Case et al., 2011; Kreke, Sharp, Lee, & Goldstein, 2008). In contrast, one study demonstrated oscillatory flow stimulated greater Ca^{2+} flickers in osteoblasts (Roy, Das, Mishra, Maiti, & Chakraborty, 2014). Similarly, using a microfluidic approach, oscillatory fluid flow was more stimulatory to osteocytes (Middleton, Al-Dujaili, Mei, Gunther, & You, 2017). In 3D, most studies utilize steady flow to stimulate osteogenesis and bone formation (Bancroft et al., 2002; Cartmell, Porter, Garcia, & Guldberg, 2003; Sikavitsas, Bancroft, Holtorf, Jansen, & Mikos, 2003; Sikavitsas et al., 2005; Zhao, Chella, & Ma, 2007), but overall, studies that utilized either steady or dynamic (oscillatory, pulsatile) have reported osteogenesis. Few studies have compared steady and dynamic flow side to side in 3D, and the results are mixed. In favor of dynamic flow, intermittent flow caused greater stimulation of osteoblasts than steady flow (Jaasma & O'Brien, 2008). Pulsatile flow appeared best for bone protein formation relative to steady flow (Sharp, Lee, & Goldstein, 2009), and pulsatile flow more strongly upregulated osteoblast production of cyclooxygenase-2

while oscillatory flow more strongly upregulated prostaglandin E2 (Jaasma & O'Brien, 2008), both important osteogenic signaling factors. Even if applying steady flow, rest periods of static or low flow are recommended to overcome cellular desensitization (Robling, Burr, & Turner, 2000), with similarly mixed results in vitro (Batra et al., 2005; Jaasma & O'Brien, 2008; Kreke, Huckle, & Goldstein, 2005; Kreke et al., 2008; Partap et al., 2010; Plunkett et al., 2010; Vance, Galley, Liu, & Donahue, 2005).

Few studies have studied dynamic versus steady or static mechanical signals on tumor cells. Static flow in 2D resulted in apoptosis of cancer cells across multiple lines (Hep3B hepatocarcinoma cells, MG63 osteosarcoma cells, SCC25 oral squamous cells and A549 carcinomic alveolar basal epithelial cells) while oscillatory flow did not (Lien et al., 2013). Pulsed magnetic forces applied to TCC-S leukemic cells with a magnetic bead increased tumor cell death both in vitro and in vivo, a response that enhances when combined with an anti-cancer therapy (Ogiue-Ikeda, Sato, & Ueno, 2003; Yamaguchi, Sato, Sekino, & Ueno, 2006). In a novel microfluidic device, when mechanically-flow osteocytes were adjacent to breast cancer cells, breast cancer extravasation was significantly reduced with mechanically-stimulated osteocytes compared to static osteocytes, though the breast cancer cells remained under static conditions (Mei et al., 2019). When considering the effects of strain, 2D stretching is typically applied acutely and held steady for a period of time with a variety of results across multiple cell types (Gao & Carson, 2016; Manome, Saeki, Yoshinaga, Watanabe, & Mizuno, 2003; McKenzie, Svec, Williams, & Howe, 2020; Panzetta, Fusco, & Netti, 2019; Riching et al., 2014). One study reported that 2D cyclic compression of breast cancer cells plated underneath an agarose gel by a platen regulated necrosis vs apoptosis, and the mode of death was sensitive to loading frequency, peak applied compressive displacement, and duration of loading bout (Takao, Taya, & Chiew, 2019). Similarly, in 3D, dynamic compression of a hydrogel with Ewing Sarcoma cells altered drug sensitivity compared to static controls, and the response was sensitive to peak strain magnitude (Marturano-Kruik et al., 2018). As mentioned previously, breast cancer cells in our scaffold altered their gene expression under dynamic compression relative to static controls, though no change in viability was observed (Lynch et al., 2013). Overall, these results emphasize that future work is needed to study the impacts of steady versus dynamic mechanical signals experimentally.

One of our goals is to delineate the individual roles of matrix deformation and fluid flow on tumor cell behavior, which is challenging as they are coupled together in the body. Our approach to achieving this goal is to correlate the estimated internal mechanical signals with biological outputs following applied perfusion and compression experimentation. By comparing the effects of perfusion alone to configurations that include deformation and interstitial fluid flow (i.e. compression alone and compression + perfusion), we expect to be able to isolate their respective effects. The asymmetry among the various mechanical signals will be a challenge in interpreting results. For example, compression alone, which best represents the in vivo mechanical environment by causing deformations and interstitial fluid flow, exhibits a phase lag between peak strains and peak wall shear stresses that may have biological implications. For our particular approach, a crucial consideration is that we use steady rather than dynamic perfusion. As shown by our computational results, this results in compression-induced flow and applied perfusion acting in concert (i.e. larger velocities) in the upper scaffold region during part of the loading cycle, leading to greater WSSs in that region. Conversely, in the latter half of the loading cycle, compression-induced flow and applied perfusion act in concert at the lower portion of the scaffold. Dynamic perfusion should be incorporated in future experiments to better reflect in vivo physiology, but mechanical signal asymmetries will still remain. Some approaches for dealing with the asymmetries could be to incorporate live imaging during loading to sense Ca²⁺ signaling (a known intracellular flow signal (Chen et al., 2000)), and/or intracellular strain signals (i.e. AP-1 (Ramani-Mohan et al., 2018)). Another approach could be to apply larger magnitudes of loading to help augment the signal-to-noise ratio in various strain- and flow-response pathways.

In summary, we have generated multiphysics models of our multimodal loading experiments to estimate interior scaffold strains and interstitial fluid velocities and wall shear stresses during loading experiments with breast cancer cells in a bone microenvironment. Our long term goal is to study bone metastatic breast cancer cell mechanobiology, and to understand how anabolic mechanical loading confers anti-tumorigenic effects to breast cancer cells (Fan et al., 2020; Lynch et al., 2013; Pagnotti et al., 2016). We confirmed that

our imposed mechanical signals are within the range known to stimulate an anabolic response in bone cells, thus our experiments will reflect conditions during anabolic loading in preclinical models of bone metastasis.

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Conflict of Interest: The authors have no conflicts to declare.

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Figure Legends:

Figure 1: In vitro multi-modal loading system with 3D bone mimetic scaffolds. (a) In future experiments, breast cancer cells will be seeded in bone-mimicking scaffolds fabricated from poly(lactide-glycolide) and bone mineral (hydroxyapatite). (b) Scaffolds will be loaded in a multi-modal bioreactor system under nine configurations of dynamic compression and direct perfusion.

Figure 2: 3D reconstruction of scaffold from microCT image and solid-fluid assembly model. (a) The bone-mimicking scaffold is highly porous with mean macropore size 432 μm and mean wall thickness 58 μm (Pathi et al., 2010). (b) A scaffold was scanned using high-resolution microCT images with voxel size 5.4 μm (Liu et al., 2018). (c) The 3D voxel model of the scaffold (shown in ivory) as well as the fluid domain (shown in cyan) was generated using ScanIP. The solid-fluid assembly was a sub-domain of the whole model, with final dimensions 1.2 mm thick and 2 mm wide.

Figure 3: Multiphysics simulation model. (a) Volumetric meshes of the fluid and solid domains were directly generated in ScanIP from 3D voxel models. (b) Fluid and solid surface meshes formed a watertight assembly along with shared nodes on the interior fluid-solid interfaces. (c) Boundary conditions were assigned to match the experimental setup. Interior fluid-solid interface was linked together in order to transfer coupled data (i.e., solid displacements and fluid forces).

Figure 4: Heat maps and distributions of surface principle strain of low and high compression. (a) Surface strain contours of 5% compression were distributed evenly in the scaffold with 70% values located in 5,000-20,000 $\mu\epsilon$ range. (b) Strains of 10% compression shifted towards higher range (10,000-40,000 $\mu\epsilon$).

Figure 5: Median WSS versus time during the 1 Hz loading cycle. WSSs induced by compression alone revealed symmetrical waveforms. However, when scaffolds were loaded with both compression and perfusion, the waveforms became asymmetric. Peak WSS values occurred at 0.25 sec of each cycle and increased with higher magnitude loading. WSS = wall shear stress.

Figure 6: Cyclic compression caused dynamic interstitial fluid flow. (a) In the C+P- simulation, velocity vectors showed fluid flow changed direction during scaffold loading (fluid perfused out) and unloading (fluid resorbed in). (b) In contrast, during combination loading (shown C+P+), fluid velocity vectors at 0.25 sec showed greater fluid flow going upward, the same as the direction of applied perfusion. Fluid flow was much lower at 0.75 sec with some reversed velocity vectors seen.





