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Communication

Upregulation of COX-2 in MCF7 Breast Cancer Cells When Exposed to Shear Stress

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Abstract

***Background*:** Invasive breast cancer affects 1 in 8 women in the United States and causes one of the highest cancer mortality rates for women. Cyclooxygenase-2 (COX-2), a central enzyme in prostaglandin biosynthesis, is implicated in breast cancer initiation, progression, invasion, and metastasis, and has been linked to the mechanotransduction of breast cancers. However, it is not currently known if shear stress mediated mechanotransduction is affected by COX-2. Therefore, in this report, we hypothesized that COX-2 modulates shear stress mechanotransduction in luminal A breast cancer cell line MCF7, causing proliferation of tumor cells and chemoresistance.

Methods: In order to probe this hypothesis, we utilized a bioreactor capable of stimulating tumor cells with variable shear stress in a 3D microenvironment and investigated the influence of shear stress stimulus on MCF7 breast cancer cells. Tumor cells were encapsulated within a hydrogel composed of agarose and collagen type I and placed under

pulsatile shear stresses of 3.04 or 3.25 dynes/cm^2. Shear stress stimulation was conducted for 24 hours and compared with the non-stimulated 3D cultured cells. Shear stresses were quantified using COMSOL finite element modeling.

Results: MCF7 cells exposed to shear stress were discovered to increase cellular area and proliferation, displaying enhanced invasive potential and cancer progression. Shear stress stimulated cells also significantly upregulated a variety of genes influencing metastasis and chemoresistance, but most significantly COX-2. Treatment with celecoxib, a selective inhibitor of COX-2, showed effective downregulation of COX-2 expression at the gene and protein level. Additionally, shear stress stimulated cells displayed enhanced resistance to paclitaxel treatment.

Conclusions: Taken together, these results demonstrate the role of COX-2 in the enhancement of breast cancer disease progression under shear stress stimulation.

Keywords

COX-2; Celecoxib; breast cancer; mechanotransduction; shear stress; 3D bioreactor; chemoresistance; proliferation; paclitaxel

1. Introduction

Breast cancer remains the number one newly diagnosed and second overall most lethal form of cancer for women in the United States [1]. The etiology and pathology of breast cancer is extremely diverse, with highly aggressive invasive breast cancers demonstrating poor clinical prognoses. The molecular mechanisms underlying the aggressive nature of certain forms of invasive breast cancers are poorly understood. Cyclooxygenase-2 (COX-2) expression is elevated in several neoplasias and are implicated to be directly involved in mammary carcinogenesis [2]. COX-2 expression holds prognostic value, being associated with significantly poor survival, enhancing a tumor-promoting microenvironment and enhanced ability to induce metastasis [3]. In fact, COX-2 inhibitors, including celecoxib, have been documented to play both a chemotherapeutic and chemo-preventive role in breast cancer [4, 5]. Furthermore, knockdown of COX-2 gene expression is documented to result in a loss of function of metastatic invasion of human breast cancer cells, with the key implication being the involvement of the COX-2 pathway in mechanotransduction [6].

In this study, we sought to test the hypothesis that shear stress within the mammary tumor microenvironment may regulate COX-2 expression, leading to metastatic traits in luminal breast cancer cells. Specific to metastatic breast cancer, an often overlooked aspect of the tumor microenvironment (TME) is the application of physiological mechanical stimuli such as compression, shear stress, stiffness, and 3D cell culture, all of which continuously influence cellular signaling and downstream effects. Breast cancer cells within the TME experience a wide range of shear stresses, including vascular blood flow, interstitial fluid flow, and fluid movement within pleural effusion [7]. These shear stress stimuli have been shown to promote cell motility, adhesion, and metastasis in breast cancer, as well as in a variety of other cancer types [8-13]. These shear stress values are predicted to be within the range of 0.1 dynes/cm^2 for interstitial flows, 0.5-30 dynes/cm^2 within the blood stream [9], and up to 18.4 dynes/cm^2 within pleural

effusions [14], though in vivo measurements remain elusive and are desperately needed. To more fully understand the influence of the physiologic shear stress stimulation on breast cancer progression, bioengineering models that consider the physiological 3D TME and mechanical stimuli are required.

To address this critical need, we utilize our previously developed shear stress bioreactor [7] to stimulate MCF7 luminal A breast adenocarcinoma cells within a highly tunable 3D microenvironment. Consistent with our previous studies, we find that breast cancer cells stimulated for 24 hours alter their morphology via an increase in cellular area and a decrease in circularity. Stimulated breast cancer cells also enhance their proliferation potential, chemoresistance, and expression levels of a variety of genes tied to metastasis, chemoresistance, and mechanotransduction. MCF7 cells upregulated the expression of COX-2, and established its role in mechanotransduction [6] via shear stress stimulation. To the best of our knowledge, this is the first investigation linking COX-2 upregulation to shear stress mechanotransduction.

2. Materials and Methods

2.1 Cell Culture

Cell culture reagents purchased from Invitrogen (Carlsbad, CA) included RPMI 1640 growth medium, antibiotic/antimycotic, type I collagen, fetal bovine serum (FBS), and 0.25% trypsin-EDTA. Human breast adenocarcinoma MCF7 cell line (HTB-22) was purchased from American Type Culture Collection (ATCC, Manassas, VA). Agarose was purchased from Boston Bioproducts Inc. (P73050G, Ashland, MA). Celecoxib (PHR1683) and paclitaxel (T7402) were purchased from Sigma-Aldrich (St. Louis, MO). AlamarBlue reagent was purchased from Fisher Scientific (Pittsburgh, PA).

MCF7 cells were cultured in 15 cm tissue culture treated polystyrene plates using RPMI 1640 growth medium supplemented with 10% FBS and 1X antibiotic/antimycotic until 80% confluency was reached. Cells were maintained routinely in tissue culture, until harvested for use in the 3D bioreactor. MCF7 cells were concentrated to 10 X 10^6 cells/mL before suspension into the agarose/collagen hydrogel. Hydrogels were polymerized as described previously [7]. Briefly, the interpenetrating network (IPN) hydrogel comprised of 3% w/v agarose and 500 µg/ml type I collagen. Hydrogels were supplemented with 10% FBS.

2.2 Immunocytochemistry

Reagents for immunocytochemistry were purchased from Invitrogen (Carlsbad, CA) and included formalin, Goat serum, Triton-X, bovine serum albumin (BSA), phosphate buffered saline (PBS), 2-(4-Amidinophenyl)-6-indolecarbamidine dihydrochloride (DAPI), Alexafluor488-conjugated phalloidin, and ProLong Gold Antifade Mountant. Anti-Ki-67 antibody was purchased from Thermo Fisher Scientific (BDB558615, Pittsburgh, PA), and COX-2 antibody (sc-376861) was purchased from Santa Cruz Biotechnology (Dallas, TX).

Once hydrogels were removed from static culture or the bioreactor, they were placed in 4% neutral buffered formalin. They were then processed for paraffin embedding and subsequently sectioned perpendicular to fluid flow stimulus. Slides were blocked for one hour (composed of 10% goat serum and permeabilized with 0.1% Triton-X) and incubated for two hours with stain specific antibody (DAPI, Alexafluor488-conjugated phalloidin, anti-Ki-67, or anti-COX-2) diluted

with formulated blocking buffer. Slides were subsequently washed with 1X PBS and DI H_2O. Finally, an antifade mounting agent was used to mount cover slides. Resulting slides were imaged with the Olympus IX81 confocal microscope (equipped with Yokogawa CSU-X1 confocal scanning laser unit, iXon x3 CCD camera, and Metamorph 7.8 software). Quantification of the resulting images was performed in ImageJ (ImageJ win64). Mean RGB values were recorded for anti-COX-2 images and normalized to DAPI values for each picture. The number of cells expressing Ki67 was normalized to the total number of cells imaged. A minimum of 5 images were taken per sample with a minimum of 3 experimental replicates analyzed per condition.

2.3 3D Shear Bioreactor

Shear bioreactor construction, description, assembly, and use were described previously [7]. Briefly, continuous cell culture medium flow was provided from a peristaltic pump at a volumetric flow rate of 2.276 cm^3/s. Cell culture medium was pumped through the inlet flow chamber and hydrogel stacks, then back out into the growth medium reservoir. The bioreactor and cell culture medium reservoir were placed into the incubator and the pump was started with a flow rate of 1.11 cm^3/s, which was then gradually increased to 2.276 cm^3/s, providing fluid flow from the bottom to the top of each hydrogel stack. Experiments were performed for either 24 or 72 hours of continuous applied shear stress. For experiments performed using celecoxib or paclitaxel, compounds were dissolved in the perfusate to final concentrations of 10 µM and 25 µM, respectively. Celecoxib inhibitor experiments were performed for 24 hours and paclitaxel drug experiments were performed for 72 hours. Each experimental condition was repeated a minimum of 3 times with 4 replicates of each shear condition within each trial. Shear stress was modeled using COMSOL Multiphysics 4.2a.

To modulate the shear stress experienced by cells within the 3D bioreactor, stainless steel rods of 5 mm or 30 mm in length were fitted into the radial flow chambers of the bioreactor in an alternating pattern (Figure S1A). Placement of these rods modulated the fluid flow velocity within the hydrogels, and thus the shear stress experienced by the respective encapsulated cells (Figure S1D).

2.4 Cell Morphology

Hematoxylin and eosin (H&E) staining was used in conjunction with ImageJ software to quantify shape factor changes, including cellular area and circularity. Images were taken at 40X magnification via an inverted color microscope.

2.5 Cell Viability - AlamarBlue Fluorescence

Cell viability of the MCF7 cells within the hydrogels was performed using alamarBlue metabolic activity. Hydrogels were removed from the bioreactor and placed in 12 individual well plates with 2 mL of 10% RPMI cell culture medium for 2 hours. One-tenth dilution of alamarBlue reagent was then added to each well and the plate was incubated for an additional 1.5 hours. After this incubation period the cell culture medium was removed, placed into 12 separate well plates and read using a Synergy HT microplate reader (BioTek Instruments, Inc. Winooski, VT) at 560 nm

excitation and 590 nm emission. The resulting fluorescence values were normalized to unstimulated 3D controls and used to describe the percentage of normalized cell viability.

Drug studies were performed on all experimental and control conditions to investigate the presence of chemotherapeutic resistance due to shear stress stimulation. A previously identified IC_{50} concentration of paclitaxel (25 μM) was used to investigate drug resistance in cells exposed to shear stress [7, 15, 16].

2.6 Gene Expression

Gene expression analysis was performed using a commercially available quantitative PCR array (PAHS-131Z, human breast cancer array, Qiagen, Valencia, CA). The commercial plate included qPCR controls including a reverse transcription control, human genomic DNA contamination control, and a positive PCR control. MCF7 cells within the collagen type I/agarose IPN hydrogels were either exposed to 24 hours of shear stress in the 3D shear bioreactor or maintained in control unstimulated 3D hydrogels submerged in cell culture medium within the incubator. Following 24 hours, total RNA was extracted from cells using the RNeasy Mini Kit (Qiagen, Valencia, CA) following the manufacturer's protocol. The NanoDrop 1000 spectrophotometer (NanoDrop, Wilmington, DE) was used to determine sample concentration and quality and all samples used in the PCR array passed RNA purity (260/280 and 260/230) with a ratio of 2.0 or better. The $2^{-\Delta\Delta CT}$ method [17] was used to determine fold changes in gene expression between MCF7 cells exposed to shear and control unstimulated cells.

2.7 Computational Analysis of Shear Stress

Computational analysis of shear stress values was previously described [7]. Briefly, material property constants were determined through direct measurement via hydrostatic water columns, SEM images, and mercury porosimetry. COMSOL Multiphysics 4.2a was utilized to represent the 3D bioreactor and evaluate shear stresses on an idealized cell (Figure S1A). A mesh analysis was conducted to confirm accuracy of shear values (Figure S1B).

2.8 Statistical Analysis

Statistical analysis and graphical plots were created in GraphPad Prism 5.0 (www.graphpad.com, San Diego, CA). All drug treatments were normalized to their respective control untreated conditions, and viability was expressed as a percentage. All data is represented as mean±SEM and are results of a minimum of 3 independent experiments.

3. Results

3.1 Hydrogel Characterization and Finite Element Model of 3D Bioreactor

The COMSOL model of the 3D bioreactor showed shear stress values within the hydrogels to be 3.25 dyne/cm^2 under 5mm rod conditions, and 3.04 dyne/cm^2 under 30 mm rod conditions (Figure S1C and Figure S1D). The variation in shear stress values was manipulated through variation in rod length within the reactor's radial flow channels. Additional ranges in shear stress stimulation can be reached by altering input peristaltic pump settings in future investigations.

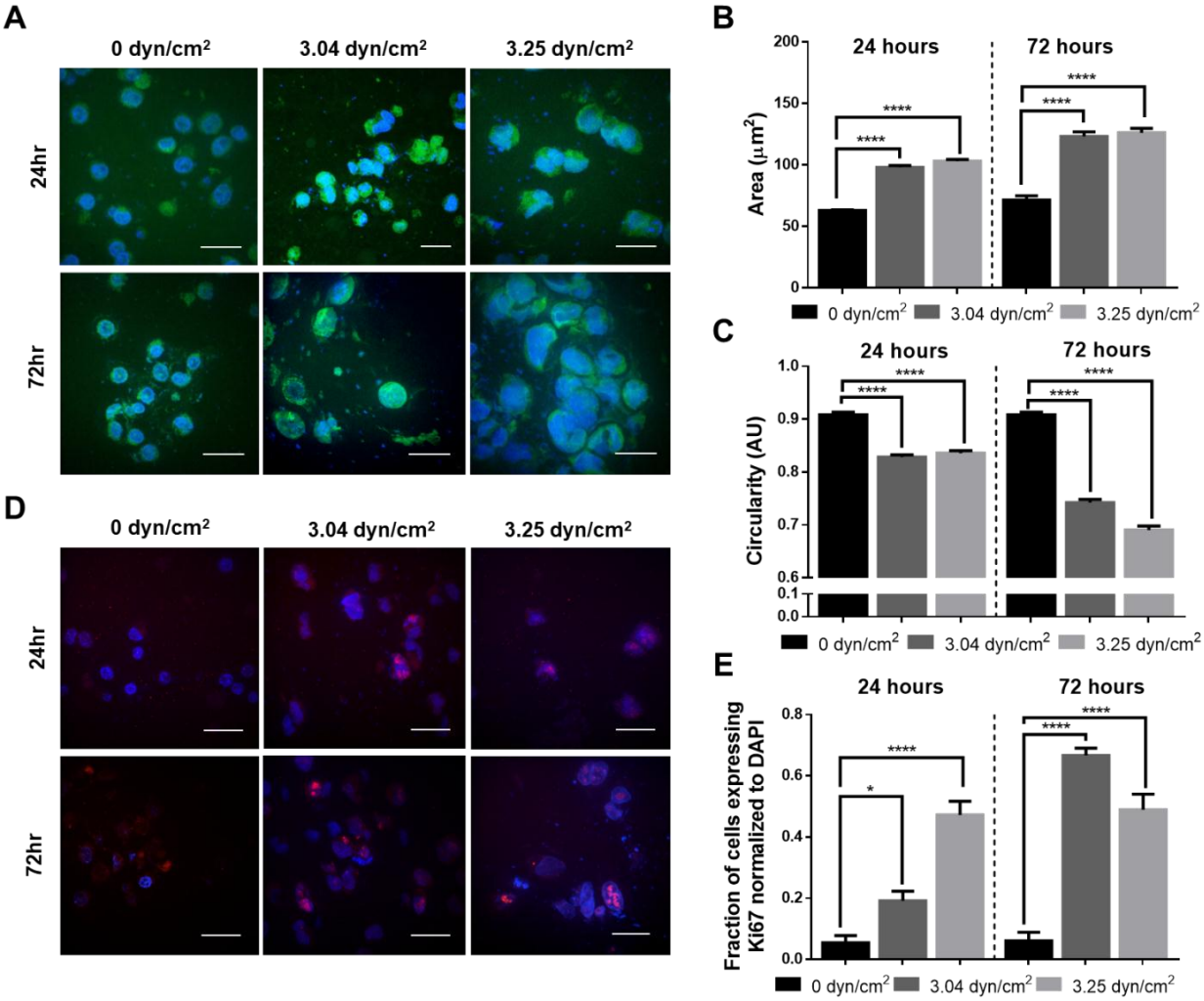

Figure 1 A. MCF7 cells were dual stained with phalloidin (green) for actin arrangement and DAPI (blue) for cell nuclei in order to qualitatively confirm the morphological changes observed in the H&E study. Representative images were captured of MCF7 control cell samples incubated for 24 and 72 hours within IPN agarose/collagen hydrogel with no shear applied and also of MCF7 cells exposed to 24 or 72 hours of shear stress (respectively 3.04 dyne/cm^2 and 3.25 dyne/cm^2). Cells in the control condition appear rounded with high circularity. Scale bar is 20 μm. **B.** Morphometric analysis and quantification of change in cellular area (μm^2) in cells stained with H&E, exposed to 24 or 72 hours of shear (3.04 dyne/cm^2 and 3.25 dyne/cm^2) as compared to control conditions. Shear areas for both 24 and 72 hours were significant when compared to no shear controls of respective shear exposure time. All values for area were significantly different when comparing 24 vs. 72 hour shear exposure (**** p< 0.0001, one-way ANOVA, n≥5). **C.** Morphometric analysis and quantification of change in cellular circularity (dimensionless) in cells exposed to 24 or 72 hours of shear (3.04 dyne/cm^2 and 3.25 dyne/cm^2) as compared to control conditions. All values for circularity were significantly different when comparing 24 vs. 72 hour shear exposure (**** p< 0.0001, one-way ANOVA, n≥5). **D.** Changes in proliferation with shear were assessed by staining MCF7 cells with the Ki67 antigen (red); nuclei were counterstained with DAPI (blue). Representative images of MCF7 control cell samples and cell samples

exposed to shear (3.04 dyne/cm^2 and 3.25 dyne/cm^2) were incubated for 24 and 72 hours respectively within IPN agarose/collagen hydrogel. More proliferation was observed under both shear conditions lasting 24 or 72 hours when compared to respective controls. Scale bar is 20 µm. **E.** Quantification and statistical comparison of cells expressing Ki67 in each condition. The fraction of cells expressing Ki67 was determined by counting cells positive with Ki67 antigen normalized to the total number of cells in that given field of view. Both shear conditions show a significant increase in the proportion of cells expressing Ki67, indicating an increased proliferative capacity with exposure to shear stress. This increase in Ki67 expression is intensified by prolonged shear exposure (**** $p<0.0001$, * $p<0.01$, one-way ANOVA, $n\geq3$).

3.2 Shear Stress Significantly Upregulates Genes Implicated in Chemoresistance, Metastasis, Invasive, and Proliferation

qRT-PCR quantified the changes in the gene expression of luminal A breast cancer MCF7 cells after stimulation with shear stress for 24 hours when compared to unstimulated controls. Seven genes were found to have a greater than two-fold increase under shear stimulation when compared to control. These genes included: *PTGS2* (Prostaglandin-endoperoxide synthase 2, *COX-2,* 3.63-fold increase), *SERPINE1* (serpin peptidase inhibitor, *PAI-1,* 3.02-fold increase), *PLAU* (Plasminogen activator, *ATF,* 2.80-fold increase), *HIC1* (hypermethylated in cancer 1, *ZBTB29,* 2.62-fold increase), *BCL2* (B-cell lymphoma 2, 2.57-fold increase), *TP53* (tumor protein 53, 2.24-fold increase), and *ABCG2* (ATP-binding cassette, *ABC15,* 2.17-fold increase). The alterations observed in these genes implicated a chemoresistant, metastatic, invasive, and proliferative phenotype of MCF7 breast cancer cells under shear stress [18-24].

3.3 Shear Stress Increases Cellular Area and Decreases Circularity of MCF7 Cells

Application of shear stress can change cell shape [25] and this morphological change can influence cell fate [26]. Therefore, circularity and area of the MCF7 cells under shear stimulation were compared to unstimulated controls. MCF7 cells experiencing shear stress exhibited morphological changes, elongating them and making them more ellipsoidal (Figure 1A).

The quantification of morphometric changes revealed a significant increase in cellular area for all MCF7 cancer cells exposed to shear when compared to controls (Figure 1B). Similar results were discovered when comparing circularity of the breast cancer cells. All shear stressed breast cells displayed a decrease in cell circularity with enhanced consequences under the longer stimulation of 72 hours compared to 24 hours (Figure 1C).

3.4 Shear Stress Significantly Increases MCF7 Proliferation

Changes in proliferation of shear stress stimulated MCF7 breast cancer cells were assessed through Ki-67 expression quantification (Figure 1D). Significantly increased Ki-67 expression was found for both 24 hours and 72 hours in shear stressed conditions, when compared to their respective controls (Figure 1E).

3.5 Celecoxib Treatment Successfully Inhibits COX-2 Overexpression Under Shear Stress

Given that COX-2 was upregulated under shear stress compared to unstimulated control, the protein expression of COX-2 was validated by immunofluorescence. MCF7 breast cancer cells exposed to shear stress had significantly higher COX-2 expression compared to the control. The enhanced COX-2 expression from shear stimulation was successfully inhibited with celecoxib treatment reducing the expression of normalized red fluorescence (Figure 2A and Figure 2B). No significant change in MCF7 breast cancer cell proliferation was observed under the celecoxib treatment subjected to 3.04 dyne/cm^2 stimulation, whereas significant reduction in proliferation was observed with 3.25 dyne/cm^2 stimulation (Figure 2D). These results indicate that celecoxib treatment is successful at inhibiting the shear stress-stimulated upregulation of COX-2 in MCF7 breast cancer cells. However, celecoxib treatment does not contribute to a reduction in MCF7 proliferation, unless stimulated at higher shear stress values.

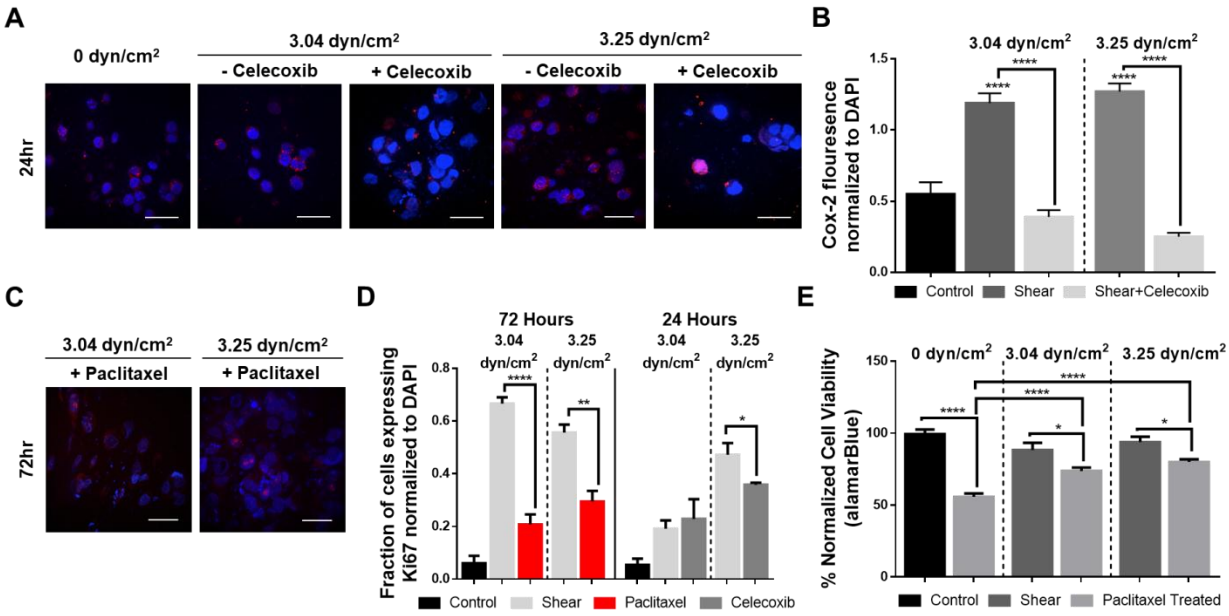

Figure 2 A. Cells were dual stained with COX-2 (red) and DAPI (blue) for cell nuclei. Representative images of MCF7 control cell samples incubated for 24 hours within IPN agarose/collagen hydrogel with no shear applied, cell samples exposed to 24 hours shear stress (3.04 dyne/cm^2 and 3.25 dyne/cm^2), and cell samples exposed to 24 hours of shear stress (3.04 dyne/cm^2 and 3.25 dyne/cm^2) with celecoxib treatment (10µM) by perfusion in media. Scale bar is 20 µm. **B.** Quantification of COX-2 expression in each 24 hour condition. COX-2 expression is significantly increased under both shear conditions as compared to control (**** p< 0.0001, one-way ANOVA). With celecoxib treatment COX-2 expression is significantly reduced as compared to the respective untreated shear condition (**** p<0.0001, one-way ANOVA). **C.** Representative image of Ki67 and DAPI stains after 72 hours of incubation with paclitaxel (25µM) treated in shear conditions (3.04 dyne/cm^2 and 3.25 dyne/cm^2). Proliferation is still seen in paclitaxel treated shear conditions but not as wide spread as control and untreated shear conditions (Figure 1D, e). Scale bar is 20 µm. **D.** Quantification of Ki67 and DAPI staining of MCF7 cells under shear stress (3.04 dyne/cm^2 and 3.25 dyne/cm^2) for 72 hours

exposed to paclitaxel and 24 hours exposed to inhibitor celecoxib, respectively. Ki67 expression was significantly reduced under both shear conditions when treated with paclitaxel. This demonstrates a reduction in proliferation (**** $p<0.0001$, ** $p<0.001$, one-way ANOVA). Proliferation was not significantly reduced through COX-2 inhibition treatment with celecoxib under 3.04 dynes/cm^2 but was when exposed to 3.25 dynes/cm^2 of shear stress (* $p<0.01$, one-way ANOVA). **E.** Quantification of cell viability using alamarBlue after 72 hours of incubation with paclitaxel (25 μM). Drug treatment significantly decreased cell viability in all experimental conditions. However, cells exposed to shear stress were more resistant to paclitaxel treatment (**** $p<0.0001$, * $p<0.01$, one-way ANOVA).

3.6 Shear Stress Stimulated MCF7 Cells are Chemoresistant to Paclitaxel

Stemming from the observed upregulation of drug resistant genes seen in the PCR array, MCF7 cells were treated with 25 μM paclitaxel for 72 hours to investigate chemoresistance potential (Figure 2C). Quantification of Ki67 expression revealed that shear stimulated and paclitaxel treated MCF7 breast cancer cells had significantly less proliferation than their non-drug treated counterparts. Paclitaxel caused successful MCF7 breast cancer cell death in the unstimulated control at an IC_{50} concentration of 25 μM and viability of 55.73±2.40%. Paclitaxel treatment on shear stress stimulated MCF7 remained significantly more viable than the drug treated no shear control (Figure 2E). These findings suggest that MCF7 breast cancer cells stimulated with shear stress are chemoresistant and can survive more effectively under paclitaxel treatment. However, under paclitaxel treatment, proliferation of the shear stimulated MCF7 breast cancer cells is reduced, supporting the drug's effectiveness at inhibiting the growth rate of the tumor.

4. Discussion

Interstitial and vascular fluid flow apply shear stress to cells in many microenvironments [9, 27, 28]. The mammary TME enhances both inflammation and vascularity in the surrounding tissue, providing an abrupt increase to interstitial flow and shear stress experienced by tumor cells [9, 29, 30]. The outward moving flow gradient results in chemoresistance by impeding drug delivery and promotes cancer cell invasion [31-34]. Shear stresses are also experienced by cells within the vasculature, such as circulating tumor cells (CTCs) that lead to distant seeding of breast cancers [9]. Within pleural effusion, one of the most common metastatic sites for mammary tumors, cells experience an even larger range of shear stresses stemming from breathing and corresponding lung movement [14, 35]. In all of these scenarios, breast cancer cells are mechanically stimulated in ways that are typically overlooked in most drug screening, drug efficacy, and other treatment studies. Therefore, we created an *in vitro* bioengineering model to understand how shear stress affects breast cancer cells in a dynamic mechanical microenvironment.

Post shear stress stimulation, breast cancer cell morphology was observed to change significantly. An increase in cellular MCF7 area and decrease in circularity was observed for all shear magnitudes and time points compared to controls. As morphological changes have been known to influence cellular fate [26] and elongation has been shown to indicate invasive potential [36], resulting levels of proliferation and cell death were investigated. Cancer cell proliferation increased under shear stress stimulation (Figure 1E) and no significant change in cell death was

observed (Figure 2E). These data indicated that the physiological levels of shear stress have a strong influence on the progression of breast cancer, through enhanced proliferation potential and thus growth of the tumor.

Although COX-2 is critical for mechanotransduction within breast cancer cells and is a known effector of migration, proliferation, and invasion [6], it is not yet known if shear stress mechanotransduction in breast cancers is mediated via COX-2. Therefore, we investigated the COX-2 levels in MCF7 cells under shear stress. Assessment of mRNA expression post shear stress stimulation revealed significant upregulation of COX-2, PAI-1, PLAU, HIC1, BCL2, TP53, and ABCG2 compared to unstimulated controls. The increase in COX-2 gene expression translated into increased COX-2 protein expression, similar to observations in other cells in response to shear [37, 38]. COX-2 expression is correlated with mechanotransduction in breast cancer cell lines [6], indicating that an increased COX-2 expression, along with an increase in expression levels of PAI-1, may represent a more invasive phenotype in MCF7 cells exposed to shear stress. Indeed, Yoon et al. reported increased force generation and metastatic ability, invasion, and cytoskeletal remodeling in MCF-7, SUM-149, and MDA-MB-231 cells with increased COX-2 expression [6]. It is not surprising, therefore, that as described in this report, inhibition of the mechanotransduction through COX-2 via celecoxib hindered one component of shear stress-induced change within MCF7 cells (Figure 2B). However, COX-2 inhibition did not result in a change in proliferative capacity of cells stimulated with 3.04 dyne/cm^2 but did significantly reduce the proliferation under the higher shear stress value of 3.25 dyne/cm^2 (Figure 2D). This reduction in proliferation via celecoxib treatment is consistent with previous findings of COX-2 inhibition on 2D monolayers of MCF7 cells [39]. These observations indicate that 3D culture and magnitude of shear stimulation may alter MCF7 proliferative response to celecoxib. The inhibition of the COX-2 pathway is highly relevant in breast cancer treatment, as it is a component of clinical treatment in combination with other chemotherapy drugs and treatment modules [40]. In future studies, the downstream effects of COX-2 modulation will be investigated in order to tie it more directly to specific shear stress mechanotransduction pathways [6].

Due to the increased expression of the drug resistant gene ABCG2 and anti-apoptotic gene BCL-2, we investigated whether breast cancer cells under shear stress showed a greater resistance to chemotherapy. Resulting cell viability studies after paclitaxel treatment showed a significant increase in viability when compared to drug treated controls. Several reports in literature suggest a causal link between COX-2 and the development of multidrug resistance via ABCG2 in breast cancer[41, 42]. COX-2 expression is also documented to induce genomic instability, BCL-2 expression, and chemoresistance in MCF7 cells [43], further attesting to the potential link between shear stress stimulation mediated COX-2 expression to the development of a chemoresistant phenotype. Therefore, shear stress stimulation and associated mechanotransduction pathways need to be considered when developing new drug therapies targeting breast cancer.

The use of this experimental model is limited in its downstream analysis, as well as its live cell interrogation during shear stress stimulation. The agarose component of the hydrogel is non-digestible by the encapsulated cells, and thus migratory and metastatic behavior cannot be visualized and quantified directly. As cells cannot yet be removed from their 3D hydrogel environment, downstream quantification through molecular biology techniques, such as in FACS analysis, is prevented. Deeper interrogation of this mechanotransduction pathway both up and

downstream of COX-2 would also be beneficial for the development of future pathway targets critical to the cellular transmission of shear stress signaling. Additional studies should be performed in similar cell lines as well as patient samples for confirmation of physiological and clinical relevance.

5. Conclusion

Through the use of a highly versatile 3D shear stress bioreactor, our results indicate that MCF7 breast adenocarcinoma cells stimulated by shear stress displayed enhanced invasive potential, proliferation, and drug resistance to paclitaxel chemotherapy. We determined COX-2 to be a key mediator, bridging the gap between mechanotransduction and the development of a malignant phenotype. Targeting COX-2 overexpression with a clinically available inhibitor, celecoxib, significantly mitigated the development of the malignant phenotype upon exposure to shear stress. Our results suggest that celecoxib or other COX-2 inhibitors can be a potent neo-adjuvant therapy for breast cancer, to improve sensitivity to chemotherapeutics. Lastly, our findings strongly demonstrate that mechanotransduction-oriented pathways play a major role in the development of aggressive breast cancer phenotypes and are an important consideration to improve chemotherapy and treatment outcomes.

Additional Materials

The following additional material is available.

1. Figure S1: Perfusion shear stress bioreactor simulation models.

Author Contributions

C.M.N. and E.N.H. designed and performed experiments, interpreted data, and wrote the manuscript. S.R. performed and interpreted experiments and edited the manuscript. G.M. conceptualized the study, designed the experiments, interpreted the data, wrote the manuscript, and provided funding. All authors read and approved the final manuscript.

Competing Interests

The authors have no conflict of interests to report.

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