Published in final edited form as:

Methods Mol Biol. 2016 ; 1341: 377–389. doi:10.1007/7651_2015_210.

APPLYING SHEAR STRESS TO PLURIPOTENT STEM CELLS

Russell P. Wolfe, Julia B. Guidry, Stephanie L. Messina, and Tabassum Ahsan*

Department of Biomedical Engineering, Tulane University, New Orleans, LA

Summary

Thorough understanding of the effects of shear stress on stem cells is critical for the rationale design of large-scale production of cell-based therapies. This is of growing importance as emerging tissue engineering and regenerative medicine applications drive the need for clinically-relevant numbers of both pluripotent stem cells (PSCs) and cells derived from PSCs. Here we describe the use of a custom parallel plate bioreactor system to impose fluid shear stress on a layer of PSCs adhered to protein-coated glass slides. This system can be useful both for basic science studies in mechanotransduction and as a surrogate model for bioreactors used in large-scale production.

Keywords

physical microenvironment; shear stress; bioreactor; embryonic stem cells; induced pluripotent stem cells; stem cell differentiation; mechanotransduction

1. INTRODUCTION

Emerging tissue engineering and regenerative medicine therapies increase the demand for generating clinically-relevant numbers of both pluripotent stem cells (PSCs) and cells derived from PSCs [1–3]. Large scale production of cells utilize bioreactors that maintain a well-mixed system by inducing the relative motion of culture medium to cells, which creates fluid shear stresses at the surfaces of cells. Several recent studies have shown that shear stress can affect pluripotent stem cell expansion and differentiation. Shear stress during PSC expansion has been shown to regulate the gene expression of pluripotent markers [4,5]. Furthermore, the application of shear stress during PSC differentiation promotes general mesodermal specification [6], as well as differentiation towards cardiac [7], hematopoietic [8,9], and endothelial [10,11] phenotypes. Given the implications of fluid shear stress on stem cell fate and the complexities and expense associated with large-scale systems, it is important to study the mechanistic effects of this physical cue in smaller, well-characterized systems.

A parallel plate bioreactor system allows the use of well-defined fluid flow profiles to induce shear stresses to cells attached to an adherent surface [10,12]. The shear stress is directly proportional to flow rate, which the researcher can control using commercially available

*Corresponding author: Tabassum Ahsan, Tulane University, Department of Biomedical Engineering, 500 Lindy Boggs Center, New Orleans, LA USA 70118, PHONE: (504) 988-6955, tahsan@tulane.edu.

low-cost pumps that are usually capable of imposing stresses that range at least an order of magnitude and are relevant to stir-based systems [13,2]. Since these closed-loop systems fit within standard laboratory incubators with CO₂ and temperature regulation, cells can be continuously exposed to shear stress for multiple days. Using such a bioreactor system (see Figure 1), the following is a protocol for differentiating PSCs for two days and then exposing them to two days of 15 dynes/cm² of fluid shear stress, conditions we have used for multiple studies [10,9,6]. Briefly, PSCs are cultured on a protein-coated glass slide and then placed within a custom assembly of parts that creates a channel above the cells over which fluid flow is directed. This assembly is then connected in series with a media reservoir, a peristaltic pump, and a pulse dampener to expose the cells to shear stresses induced by steady laminar fluid flow. After treatment, cells can be further cultured, passaged, or assessed for gene and protein expression. While the details below are for a specific protocol with PSCs, various experimental parameters can be changed and alternate cellular phenotypes can be used.

2. MATERIALS

As a protocol that uses a custom bioreactor system, the materials listed below include both commercially available and custom manufactured parts.

2.1 Equipment

1. Chemical fume hood
2. Laminar flow hood
3. Autoclave
4. Vacuum aspiration system
5. Peristaltic pump (Masterflex, cat. no. 7551)
 - a. 8-channel, 4-roller pump head (Cole-Parmer, cat. no. HV-07519-06)
 - b. 4 large cartridges for L/S 25 tubing (Cole-Parmer, cat. no. HV-07519-70)

2.2 General Reagents

1. 10N sulfuric acid (see Note 1)
2. 70% ethanol: 70ml ethyl alcohol 200 proof to 30ml of water
3. Dehydration alcohol
4. Sodium bicarbonate

¹Safe laboratory procedures should always be used. 10 N H₂SO₄ is a strong acid and should be kept in a labeled acid resistant container inside the chemical hood or certified cabinet. To dispose of H₂SO₄, first neutralize with 1.7 g of sodium bicarbonate (NaHCO₃) per gram of acid and then pour into an appropriate waste container.

2.3 General Supplies

1. 6 acid resistant containers with lids (minimum of 75 mm tall)
2. Slide forceps (EMS, cat. no. 78335-35A)
3. Hemostats
4. Sterile glass Pasteur pipettes
5. Absorbent pad with plastic backing (Daigger, cat. no. EF8313AA)
6. 28mm polyethersulfone 0.2um filter (VWR, cat. no. 28200-042)
7. Instant sealing sterilization pouches
 - a. Small: 5 ¼ × 10 inch (Fisherbrand, cat. no. 01-812-54)
 - b. Medium: 7 ½ × 13 inch (Fisherbrand, cat. no. 01-812-55)
 - c. Large: 12 × 18 inch (Fisherbrand, cat. no. 01-812-58)

2.4 Culture Reagents

1. Dulbecco's phosphate buffered saline (DPBS 1X)
2. 50 µg/ml collagen type IV solution: 1 mg collagen type IV (BD Biosciences, cat. no. 354233) in 20 mls 0.05M HCl (*see Note 2*)
3. Culture medium (*see Note 3*)
4. Differentiation medium (*see Note 4*)

2.5 Culture Supplies

1. 75 × 38 mm micro glass slides (Corning, cat. no. 2947)
2. Disposable cell lifters (Fisherbrand, cat. no. 08-100-240)
3. Plastic dishes
 - a. 150 mm bacterial culture petri dishes (Corning, cat. no. 430597)
 - b. 100 × 15mm square sterile Petri dishes with lid (Thermo Scientific, cat. no. 4021)
4. 10 × 13 inch metal trays

²The procedure described here is to differentiate the PSCs for 2 days on collagen type IV and then apply shear stress for 2 days at 15 dynes/cm² as previously published [10]. In other studies we have differentiated for 1-3 days on various protein substrates for a range of shear stress magnitudes [9,6].

³Various medium formulations can be used to culture PSCs. The formulation used in our lab is currently Dulbecco's Modification of Eagles Medium supplemented with 15% ES-qualified fetal bovine serum, 2mM L -glutamine, 0.1mM non-essential amino acids, 1,000 U/mL Leukemia Inhibitory Factor, and antibiotics.

⁴PSCs can be differentiated using a variety of medium formulations, which may be specific to the study. One formulation commonly used in our lab is Minimum Essential Alpha Media supplemented with 10% fetal bovine serum, 0.1mM beta-mercaptoethanol, and antibiotics. For a study with 4 experimental samples and 4 static controls, 1200 ml of differentiation medium is needed.

2.6 Bioreactor Loop

1. Tubing
 - a. Norprene Masterflex L/S 25 tubing (Cole Palmer, cat. no. 06402-25)
 - b. Silicone tubing 0.125" ID, 0.250" OD (Cole Palmer, cat. no. 06411-67)
2. Polypropylene luers (all from Value Plastics)
 - a. Male luer integral lock ring to 200 series barb, 3/16" (cat. no. MTLL250-6)
 - b. Female luer tread style to 200 series barb, 1/8" (cat. no. FTLL230-6)
 - c. Female luer thread style coupler (cat. no. FTLLC-6)
 - d. Female luer thread Style with 5/16" Hex to 1/4-28 UNF Thread (SFTLL-6)
3. Pulse dampener, 190 ml dead vol, max 60 PSI (Cole-Parmer, cat. no. EW-07596-20)
4. Media bottle: Nalgene PC 250 ml (Cole-Parmer, cat. no. EW-00091-HX)
5. Media bottle cap: Nalgene filling/venting cap for 1/4" tubing (Cole-Parmer, cat. no. EW-06258-10)

2.7 Bioreactor Flow Chamber

1. The parallel plate chamber consists of multiple custom machined parts (*see* Figure 2A)
 - a. Top aluminum frame
 - b. Bottom aluminum frame
 - c. Flow block
2. Additional parts are cut from stock materials (*see* Figure 2B)
 - a. Rubber gasket: FDA silicon rubber, 1/8" thick, 50A durometer (McMaster, cat. no. 86045K13)
 - b. Spacer: shim, 0.020" (~0.5 mm) thickness (Precision Brand, cat. no. 44160)
3. Philips truss 2" machine screws (McMaster-Carr, cat. no. 91770A205)
4. Philips screwdriver

3. METHODS

3.1. Initial Preparation of Individual Bioreactor Pieces

1. Further customization of the flow block:
 - a. Create a smooth bottom surface by sanding under running water for 5 minutes with #220 grit paper followed by an additional 5 minutes with #600 grit paper.
 - b. Teflon tape should be wrapped around female luers and attached to the four ports (two on the side and two on top).
 - c. A 7" piece of silicon tubing should be capped with male luers on each end and then fastened to the top ports of the flow block (*see Note 5*).
2. The media bottle cap needs to be modified to include tubing to the top ports. Silicon tubing approximately 27" in length should be capped with luers and press-fit onto the cap top to create a source channel (male luer), a return channel (female luer), and a venting port (male luer).
3. The dampener needs to be customized by attaching one set of the provided barbs to the ports on the side per the manufacturer's instructions. The selected barb size should be one that tightly fits the silicon tubing. Attach 5" pieces of silicon tubing to each, capping the other end of the tubing with a male luer on the other end (*see Note 6*).
4. The spacer needs to be cut to have an inner channel with a width of 2.65 cm. The length of the channel needs to span across the slits on the flow block when aligned with the spacer.
5. A relief should be cut (size not critical) in the middle of the gasket to help avoid cracking the glass slide from the pressure of the screws.

3.2 Acid Washing of Slides

1. These steps need to be performed in chemical fume hood using standard laboratory safety procedures.
2. Partially fill three acid resistant containers with 10 N sulfuric acid, two containers with 70% ethanol, and one container with dehydration alcohol. Allow enough space so that there is no overflow when a glass slide is put into the container.
3. Using slide forceps, hold the slide and immerse it in the first container of 10 N sulfuric acid.

⁵The passage of air bubbles over adherent cells can cause cell death. The loop on the top of the flow block acts as a trap for air bubbles, which will rise into the loop. During the bioreactor setup, a binder clip is clamped to the loop to ensure that the medium will flow over the surface of the cells (the path of least resistance).

⁶The manufacturer suggests using Teflon tape when securing the barbs to the dampener. In our lab we apply gasket sealant at the base of the barb for additional protection from leaks.

4. Allow the slide to soak for approximately five seconds before transferring to the next container.
5. After letting the slide sequentially soak in each of the three containers of sulfuric acid, submerge the slide in 70% ethanol. This will help remove excess acid. Move to a second container of 70% ethanol for additional washing.
6. Briefly dip the slide into dehydration alcohol before placing the slide in a 150 mm petri dish.
7. Rinse the slides with sterile DPBS and allow slides to fully dry before autoclaving.
8. Place 3–5 slides in small sterilization pouches (avoid overlapping the slides), seal the pouch, and autoclave (*see* Note 7). Eight slides will be needed for a standard trial with 4 experimental shear samples and 4 static controls.

3.3 Bioreactor Loop Sterilization

1. Assemble a tubing loop by connecting the luers in the following order:
 - a. Source channel of cap attached to media bottle
 - b. 20" section of silicon tubing capped by a female luer on each end
 - c. 7" Masterflex L/S 25 tubing capped by a male luer on each end
 - d. 20" section of silicon tubing capped by a female luer on one end and a male luer on the other end
 - e. Dampener
 - f. 7" section of silicon tubing with a female luer on one end and a male luer on the other
 - g. Female luer coupler
 - h. 27" section of silicon tubing with a male luer on each end feeding back into the return channel of the media bottle cap
2. Tighten all connections.
3. Before placing a bioreactor loop into a large sterilization pouch, unscrew the cap from the bottle to allow airflow during the autoclave cycle. Be sure tubing does not lie across the dampener (*see* Note 8).

⁷Settings for sterilization of dry items can vary by manufacturer and model of autoclave. Many wall mounted steam autoclaves have a "gravity cycle" of 20 minutes of sterilization time at 121.0°C followed by 20 minutes of drying time, which is appropriate for all the solid bioreactor components except for the spacers.

4. Seal the pouch and autoclave the bioreactor loop. For a single pump trial, 4 loops are needed.

3.4 Flow Chamber Sterilization

1. Place the rubber gasket inside of the bottom aluminum frame and slide into the medium sterilization pouch.
2. Lay the flow chamber on its side and slide it into the pouch so that the flat surface faces away from the aluminum frame (*see* Note 8).
3. Slide the top aluminum frame into the pouch and seal the bag.
4. For a single pump trial, 4 similarly prepared chambers are needed. Autoclave the flow chambers.
5. Put two metal trays in sterilization pouches, seal, and autoclave.

3.5 Protein Coat Glass Slides

1. Using aseptic technique in a laminar flow hood, use sterile slide forceps to move each sterile glass slide into a separate sterile square dish.
2. Pipette 2 ml of the collagen type IV solution onto each slide to coat at a density of 3.5 $\mu\text{g}/\text{cm}^2$.
3. Use a cell lifter to spread the solution across the entire top of the slides, leveraging capillary action to keep the solution from wicking off the surface (*see* Note 9).
4. Eight slides will be needed for a standard trial with 4 experimental shear samples and 4 static controls.
5. With the lids on the dishes, allow the slides to sit undisturbed at room temperature for 1 hour in the laminar flow hood.

3.6 Seed Cells onto Slides

1. Using standard pluripotent stem cell culture techniques, generate a well-mixed cell suspension of 285,000 cells/ml.
2. Use a vacuum aspiration system and Pasteur pipettes to remove the collagen type IV solution from the glass slides.
3. Coat the surface of the glass slides with 1ml of the cell suspension solution for an initial seeding density of 10,000 cells/ cm^2 . Use a cell lifter to help spread the solution across the entire surface of the slides.

⁸When packing items to be autoclaved, it is important to be aware that contact between certain materials can cause deformation at such high temperatures. During autoclaving for this protocol, it is important that the aluminum frames not contact the bottom of the flow block and the Masterflex tubing not contact the dampener.

⁹When the slides are being coated with proteins or seeded with cells, it is important that the solution spreads across the top surface and does not wick over the edges. Otherwise, the slides will not be covered with the target protein concentration or cell density. After protein coating the slides, it is important to thoroughly aspirate the solution off the surface to help keep the subsequent application of cell solution from wicking.

4. Cover the petri dishes and allow the cells to attach at room temperature for 1 hour in the laminar flow hood.
5. Add 25 ml of pre-warmed (to 37°C) differentiation media to each dish and transfer dishes to an incubator set at 37°C and 5% CO₂.
6. Allow the cells to differentiate on the slides for 2 days.

3.7 Bioreactor Setup and Pre-warming

1. Lay down absorbent pads within the cell culture incubator to protect from any accidental system leaks.
2. Place the pump, complete with pump head and cassettes, within the incubator. The power cord can connect to either the built-in power socket available in some incubators or be threaded through the filter port in the back of most incubators. Depending on the incubator configuration, occasionally one or more shelves may need to be removed.
3. In the laminar flow hood, open a sterile bioreactor loop. Attach a filter to the open venting port on the media bottle cap. Tighten all connections as some may have loosened during autoclaving.
4. Add 125 ml of differentiation medium to the media bottle and close with the cap.
5. Engage the bioreactor loop with the pump by placing the cassette over the Masterflex tubing section. Push the cassette to the back of the pump head to allow room for the remaining cassettes. Make sure the media bottle is stable in a standing position and that the tubing and dampener are flat on the incubator shelf.
6. Repeat steps 1–5 for all four loops.
7. Start the pump and set to 48 RPM (*see Note 10*).
8. Add 125 mls to each of four 150 mm sterile petri dishes and place in the incubator adjacent to the bioreactor systems.
9. Let the bioreactor loops and medium warm in the incubator for a minimum of 2 hours.
10. Sterilize the spacers by placing each in an open petri dish in a closed laminar flow hood with the UV light turned on. Sterilize each side with the UV light for at least 30 minutes, using sterile slide forceps to flip over the spacer. When complete, transfer all the spacers to a single sterile 150 mm petri dish.

¹⁰Shear stress for this system is calculated by the formula $\tau = 6Q\mu/bh^2$, where b is the channel width, h is the channel height, μ is the viscosity of the medium, and Q is the fluid flow rate. The values for b and h are set by the dimensions of the spacer and in our system are 2.65 cm and 0.05 cm, respectively. The viscosity for our differentiation medium formulation is 0.012 dynes*sec/cm². The flow rate Q is set by the pump, which at 48 RPM results in 15 dynes/cm² of fluid shear stress for the parameters in this protocol.

3.8 Application of Steady Laminar Shear Stress

1. Stop the pump. Remove all four closed loops from the incubator and place on a clean lab bench lined with an absorbent pad.
2. Inside the laminar flow hood, place the Phillips screwdriver, the Phillips screws, 1 pair of autoclaved slide forceps, 2 pairs of autoclaved hemostats, and 2 autoclaved metal trays.
3. Open the two autoclaved metal trays. Maintain a sterile surface by opening one metal tray and placing it off to the side. Upon the sterile tray, place the open sterile forceps. Create a clean assembly surface by opening the second metal tray and placing it directly in front of you within easy reach. Place the 2 open hemostats on the assembly surface.
4. Bring into the laminar flow hood the dish with all the sterile spacers, 1 autoclaved flow chamber, 1 previously warmed 150 mm dish with medium, and 2 square dishes with cultured PSCs on glass slides.
5. Remove the bottom aluminum frame with gasket from the sterilization pouch and place on the clean assembly surface.
6. Using the sterile slide forceps, grab a PSC-seeded glass slide and place on the gasket within the bottom aluminum place. Take care to not disturb the top surface seeded with cells.
7. Grab a sterile spacer and place on the glass slide. Use the internal edges of the bottom aluminum frame to align the space with the slide to ensure minimal contact with cells.
8. Using the tubing loop atop the flow block as a handle, remove the flow block from the sterilization pouch and place atop the spacer. Grab the top aluminum frame and place on the flow block.
9. Using the screwdriver and 6 Philips screws, tighten the flow chamber assembly. Once the screws engage such that all components are touching, take care to evenly tighten the screws to avoid cracking the slide. Tighten sufficiently to avoid leaks.
10. Move one bioreactor loop into the laminar flow hood and open. Place the tubing so that the section with the female coupler lies across the assembly surface.
11. Clamp a hemostat on the tubing on each side of the female coupler. Remove the female coupler and attach each open end of the tubing to the flow chamber assembly.
12. Return the fully assembled bioreactor system to the incubator and engage with the pump. Remove the hemostats and start the pump (preset to 48 RPM).
13. Monitor the fluid flow to make sure it passes across the glass slide and then clamp the top loop of the flow chamber with a binder clip.

14. Place the remaining (of the two in the laminar flow hood) PSC-seeded glass slide into the 150 mm dish with medium. Place the sample in the incubator adjacent to the bioreactor systems to act as a static control.
15. Repeat steps 4–14 until all the bioreactor systems and static controls have been setup. A standard trial consists of 4 experimental shear samples and 4 static controls.

3.9 Remove Samples for Analysis

1. Stop the pump while you remove one of the bioreactor systems, then resume flow to the remaining systems. Place the removed system in the laminar flow hoods if it is necessary for subsequent sterile culture of the cells, else place on the lab bench.
2. Disassemble the bioreactor system by first clamping the tubing on each side of the flow chamber with hemostats. Unscrew the flow block from the tubing. Replace the female coupler to reassemble the bioreactor loop.
3. Use the screwdriver to evenly untighten and remove the screws. Place the top aluminum frame and flow block to the side. Remove the spacer with forceps without disrupting the cell layer on the glass slide.
4. Remove the glass slide with cells from the flow chamber assembly. Retrieve one static control sample from the incubator. Trypsinize, lyse, or culture the experimental and control samples using standard cell and molecular biology techniques as appropriate for your experimental design (see Figure 3).
5. Repeat steps 1–4 for the remaining samples.
6. Wash the flow chambers and bioreactor loops with running water before storing.

Acknowledgments

This work was funded by the NIGMS of the NIH (#P20 GM103629). Additional support was also received from the Tulane Undergraduate Research Fund (JG) and the Louisiana Board of Regents (SM & RW).

References

1. dos Santos FF, Andrade PZ, da Silva CL, Cabral JM. Bioreactor design for clinical-grade expansion of stem cells. *Biotechnol J*. 2013; 8(6):644–654. DOI: 10.1002/biot.201200373 [PubMed: 23625834]
2. Kehoe DE, Jing D, Lock LT, Tzanakakis ES. Scalable stirred-suspension bioreactor culture of human pluripotent stem cells. *Tissue engineering Part A*. 2010; 16(2):405–421. DOI: 10.1089/ten.TEA.2009.0454 [PubMed: 19739936]
3. Want AJ, Nienow AW, Hewitt CJ, Coopman K. Large-scale expansion and exploitation of pluripotent stem cells for regenerative medicine purposes: beyond the T flask. *Regen Med*. 2012; 7(1):71–84. DOI: 10.2217/rme.11.101 [PubMed: 22168499]
4. Gareau T, Lara GG, Shepherd RD, Krawetz R, Rancourt DE, Rinker KD, Kallos MS. Shear stress influences the pluripotency of murine embryonic stem cells in stirred suspension bioreactors. *Journal of tissue engineering and regenerative medicine*. 2012; doi: 10.1002/term.1518

5. Toh YC, Voldman J. Fluid shear stress primes mouse embryonic stem cells for differentiation in a self-renewing environment via heparan sulfate proteoglycans transduction. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology*. 2011; 25(4):1208–1217. DOI: 10.1096/fj.10-168971 [PubMed: 21183594]
6. Wolfe RP, Leleux J, Nerem RM, Ahsan T. Effects of shear stress on germ lineage specification of embryonic stem cells. *Integr Biol (Camb)*. 2012; 4(10):1263–1273. DOI: 10.1039/c2ib20040f [PubMed: 22968330]
7. Sargent CY, Berguig GY, Kinney MA, Hiatt LA, Carpenedo RL, Berson RE, McDevitt TC. Hydrodynamic modulation of embryonic stem cell differentiation by rotary orbital suspension culture. *Biotechnology and bioengineering*. 2010; 105(3):611–626. DOI: 10.1002/bit.22578 [PubMed: 19816980]
8. Adamo L, Naveiras O, Wenzel PL, McKinney-Freeman S, Mack PJ, Gracia-Sancho J, Suchy-Dicey A, Yoshimoto M, Lensch MW, Yoder MC, Garcia-Cardena G, Daley GQ. Biomechanical forces promote embryonic haematopoiesis. *Nature*. 2009; 459(7250):1131–1135. DOI: 10.1038/nature08073 [PubMed: 19440194]
9. Wolfe RP, Ahsan T. Shear stress during early embryonic stem cell differentiation promotes hematopoietic and endothelial phenotypes. *Biotechnol Bioeng*. 2013; 110(4):1231–1242. DOI: 10.1002/bit.24782 [PubMed: 23138937]
10. Ahsan T, Nerem RM. Fluid shear stress promotes an endothelial-like phenotype during the early differentiation of embryonic stem cells. *Tissue Eng Part A*. 2010; 16(11):3547–3553. DOI: 10.1089/ten.TEA.2010.0014 [PubMed: 20666609]
11. Zeng L, Xiao Q, Margariti A, Zhang Z, Zampetaki A, Patel S, Capogrossi MC, Hu Y, Xu Q. HDAC3 is crucial in shear- and VEGF-induced stem cell differentiation toward endothelial cells. *J Cell Biol*. 2006; 174(7):1059–1069. jcb.200605113 [pii]. DOI: 10.1083/jcb.200605113 [PubMed: 16982804]
12. Levesque MJ, Nerem RM. The elongation and orientation of cultured endothelial cells in response to shear stress. *Journal of biomechanical engineering*. 1985; 107(4):341–347. [PubMed: 4079361]
13. Cormier JT, zur Nieden NI, Rancourt DE, Kallos MS. Expansion of undifferentiated murine embryonic stem cells as aggregates in suspension culture bioreactors. *Tissue engineering*. 2006; 12(11):3233–3245. DOI: 10.1089/ten.2006.12.3233 [PubMed: 17518637]

Figure 1. Bioreactor System

The bioreactor system (**left**) uses a peristaltic pump to recirculate medium from a bottle, through a pulse dampener, across a flow chamber, and back again (**schematic in middle**). The flow chamber is an assembly of a flow block above a spacer that sits on a glass slide, all held together by a top and bottom aluminum frame with a gasket to prevent leaks (**schematic on left**).

Figure 2. Pictures of Flow Chamber Components

(A) The custom machine parts include (from left to right) a top aluminum frame, a bottom aluminum frame, and a flow block. (B) The items cut from stock materials include (left) the spacer, for which the cut width is b and the thickness is h in the shear stress calculations, and (right) the gasket, with a slit in the middle to act as a relief that helps cracking of the glass slide.

Figure 3. Phase Images of Samples

Shown are representative low and high magnification images of pluripotent stem cells from static controls and samples that were exposed to shear stress.