SUPPLEMENTARY INFORMATION

A pumpless microfluidic culture system to model the effects of shear flow on physiological barriers

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Supplemental Materials & Methods

Rocker plate

To control fluid flow in the VitroFlo plate, a custom, 3D printed, programmable rocker plate was manufactured. The programs consist of a CSV file containing time points and target angles, as described in the supplemental information. The rocker plate then calculates the angular speed during each program segment based on the current angle, the target angle and the time allotted for that specific step. The programs used in this work start at a minimum angle where the inlet reservoir is filled and the outlet is almost empty and the height difference, *dh*, is zero. In the second step, the rocker rapidly moves to a starting angle which establishes the target *dh* for the current program (*dh* determines flow and shear stress). In the third step, the rocker moves slowly to match the flow and maintain the pressure head established in step two. At the end of step three, the rocker has reached the maximum angle and quickly moves back to the initial, minimum angle, allowing the cell culture media to move from outlet to inlet through the back channel. At the minimum angle, the rocker waits for *dh* to once more reach zero and then proceeds through the cycle again.

Rocker program

The flow in the VitroFlo plate is powered by a 3D printed, programmable rocker plate made in-house. By varying the angles and angular velocity, the flow rate can be controlled. The programs used in this work follow the behaviour outlined in **Table S1**. The actual values are shown in **Table S2**. The time and angle set points are transferred to the rocker in a .csv file. When the rocker starts, it will move to the minimum angle and then start following the instructions. The time set point denotes when the corresponding angle should be reached. Based on the current position, the end position and the time information, the rocker plate then calculates its angular velocity. When the rocker reaches the end of the program, it starts over. For instance, for the 1.5 dyn/cm^2 program, the final step is to wait for 2.2. s at -34.4° (backflow). Once this step is completed, the rocker will move to -27.7° over 1.65 s to establish the pressure head that drives the flow.

Shear stress, experimental

To determine the shear stress experienced by cells cultured in the VitroFlo plate, four devices in a single 2D plate (top channel only, no membrane or bottom channel), were filled with 6 mL de-ionized water stained with food colouring. A rocker plate was reset to the minimum angle and paused. The VitroFlo plate was placed on top and the flow was allowed to equilibrate the height difference before the experiment started. Once the height difference between inlet and outlet reservoirs reached 0, the rocker plate was started. The rocker ran multiple cycles for each program and the procedure was captured using a Nikon D5300 DSLR camera. Between each program and separate movie acquisition the experiment was reset by resetting the rocker plate to the minimum angle and waiting for the height difference to equilibrate before the program was started.

The movies captured were imported into Fiji [1] using the FFMPEG plugin, cropped to single forward or back cycles and down sampled to ~100 frames per cycle. The fluid level in inlet and outlet reservoir pairs were then tracked manually using the manual tracker plugin (available at: https://imagej.net/Manual_Tracking) and used to calculate the height difference, *dh*, between inlet and outlet.

The obtained *dh* was used to calculate the flow, *Q* (equation (1)), based on the pressure difference, *dP* (equation (2)), and the channel resistance, *R* (equation (4)). Using equation (5)-

(7), the wall shear stress, τ_w, across the floor is obtained. Here η is the viscosity of the media; *w*, *h*, and *L* are the channel width, height and length; m and n are empirically determined constants valid for channel aspect ratios *h/w* < 1/3 [2].

$$Q = \frac{dP}{R} \tag{1}$$

$$dP = dh \cdot \rho \cdot g \tag{2}$$

$$Q = \frac{dh \cdot \rho \cdot g}{R} \tag{3}$$

$$R = \frac{12 \cdot \eta \cdot L}{wh^3 \left(1 - 0.63 \frac{h}{w}\right)} \tag{4}$$

$$\tau_w = \frac{2 \cdot \eta \cdot Q}{wh^2} \left(\frac{m+1}{m}\right) (n+1) \tag{5}$$

$$m = 1.7 + 0.5 \left(\frac{h}{w}\right)^{-1.4} \tag{6}$$

$$n = 2 \tag{7}$$

Shear stress, analytical

An analytical expression for the height difference, *dh*, between inlet and outlet reservoirs based on inlet volume and rocker plate angle was derived by dividing the reservoir volumes into composite volumes and calculating the fluid heights as described in the supplementary information. By using the known start angle, the known total volume of the system and setting *dh* = 0, the initial inlet reservoir volume could be calculated.

A custom Python script was used to iterate over different angles based on the rocker plate program. During each iteration, the flow was calculated based on the current angle and inlet volume using Equations (1)-(4). The flow was used to update the inlet volume before the next iteration. At the end of the iterations, lists containing *dh* and flow as functions of time were obtained. The flow was then used to calculate shear stress using Equations (5)-(7).

References

1. J. Schindelin, I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J. Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak and A. Cardona, *Nat Methods*, 2012, **9**, 676-682.
2. E. W. Young and C. A. Simmons, *Lab Chip*, 2010, **10**, 143-160.

Table S1 Description of step functions for rocker plate programs.

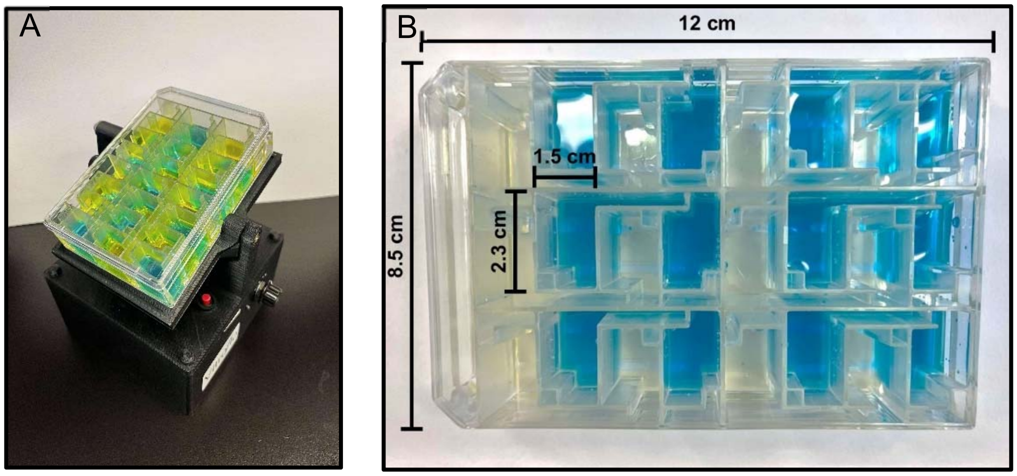
Step	Description	Purpose
0	Minimum, stationary	Cycle start; flow = 0; majority of fluid in inlet
1	Start angle, quick jump	Quick move from minimum, establish pressure head
2	Start angle to end angle, slow continuous movement	Slowly tilt rocker plate at an angular velocity matching the flow rate so the pressure head is maintained for continuous, stable flow. This step sets the flow rate for the program.
3	End angle, maximum positive	End of forward cycle
4	Quickly flip from end angle (maximum) to minimum angle	Transition to backflow; Tilting the Vitroplate back allows fluid to run over backflow wall and replenish the inlet reservoir.
5	Backflow, rocker at minimum angle, pause	Pause until outlet reservoir is drained and inlet is refilled. Height difference goes to 0
0	Cycle restarted	Height difference has reached 0, cycle restarts

Table S2: Angle and time set points for standard rocker programs in this work. Angles are given in degrees where 0 is horizontal, negative angles signify the rocker plate tilting toward the VitroFlo plate's inlet reservoirs and positive angles signify tilt toward the outlet reservoirs. The time set points instruct the length of time for the rocker to should reach the target angle. The rocker then calculates its angular velocity based on this time interval, its end position and it current position. The column "Step" shows the corresponding steps in **Table S1**. When the rocker program reaches the end, it starts over, e.g. for Program 1, once the program has waited for 2.2 s at -34.3°, it will move to -27.7° over 1.65 s.

	1.5 dyn/cm^2 20 min		**3 dyn/cm^2** 9 min		**5 dyn/cm^2** 200 s		**10 dyn/cm^2** 65 s	
Step	**Time [s]**	**Angle [°]**	**Time [s]**	**Angle [°]**	**Time [s]**	**Angle [°]**	**Time [s]**	**Angle [°]**
0 → 1	1.65	-27.7	1.65	-23.0	1.65	-16.5	1.65	-3.64
2 - 3	1200	24.5	540	24.5	200	24.5	65	24.5
4	1.65	-34.3	1.65	-34.3	1.65	-34.3	1.65	-34.3
5 → 0	2.2	-34.3	2.2	-34.3	2.2	-34.3	2.2	-34.3

Table S3: Angle and time set points for rocker program used in Caco-2 gut barrier modeling experiments.

	0.02 dyn/cm^2 15 hours	
Step	**Time [s]**	**Angle [°]**
0 → 1	1.65	-27.7
2 - 3	54000	24.5
4	1.65	-34.3
5 → 0	2.2	-34.3



C	Length (cm)	Width (cm)	Height (cm)
Plate	12.5	8.5	3.0
Reservoir	1.5	2.3	3.0
Top channel	2.5 (from inlet to outlet)	0.2	0.028
Bottom channel	5.2 (from inlet to outlet)	0.2	0.028
Backflow channel	N/A	N/A	2.5

Fig. S1 Photographs and dimensions of the VitroFlo plate. (**A**) Photograph of VitroFlo plate with lid on a rocker, showing pairs of reservoirs with blue or yellow liquid that make up 12 independent wells. (**B**) Top view of VitroFlo plate with the external dimensions of the plate and the dimensions of an individual reservoir indicated. (**C**) Summary of dimensions of features of the VitroFlo plate. Note that the channel lengths indicated here include the inlet and outlet portions; the top channel length with porous membrane is 2.0 cm, as described in the main text.

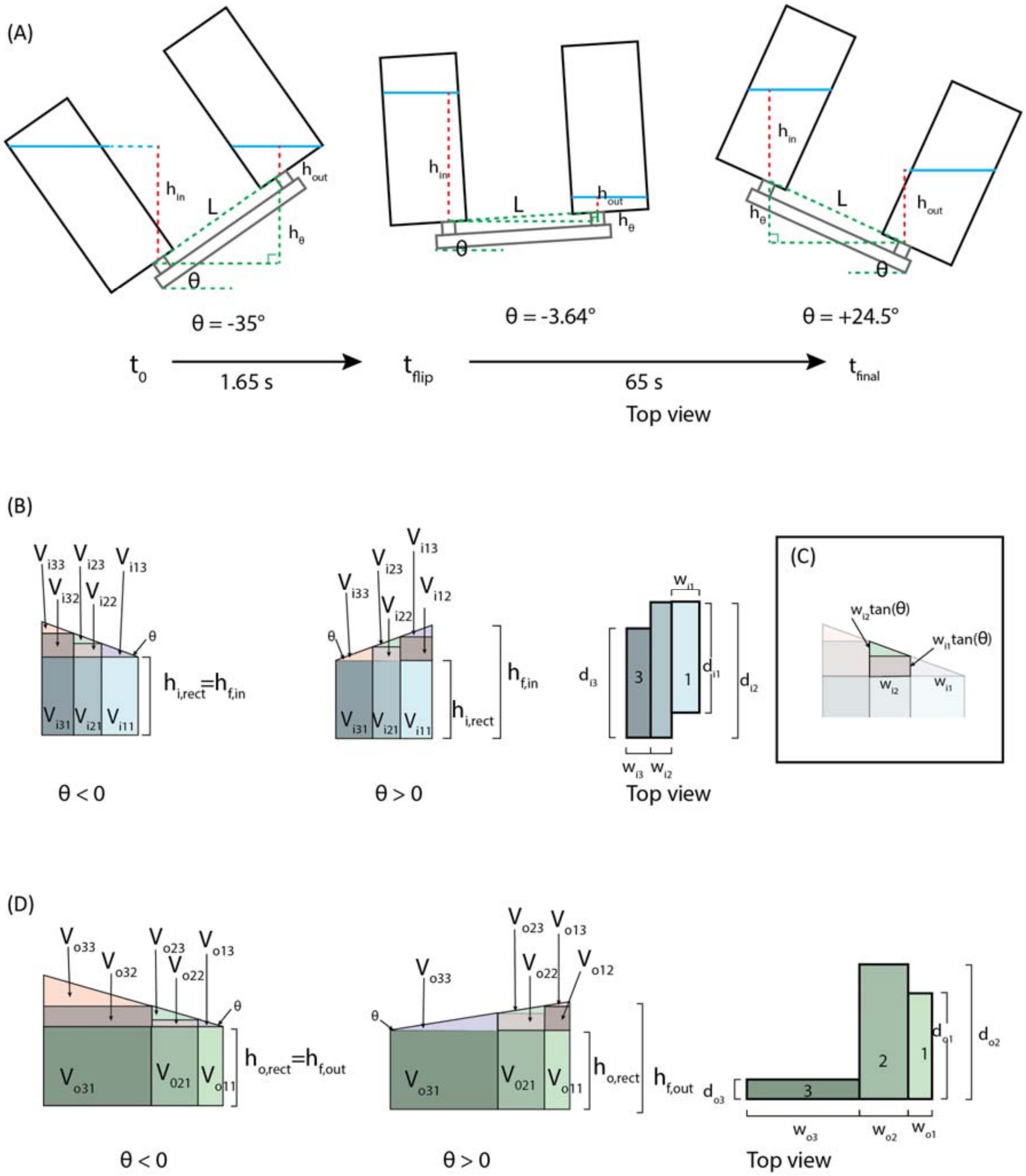


Fig. S2 Derivation of analytical expression for shear stress as a function of rocker angle and initial volume in inlet. (**A**) General problem description showing how the height difference between the reservoirs (*dh*) can be obtained by calculating the height as a function of volume for both inlet and outlet reservoirs as well as the height contributed by the rocker angle, θ. (**B**) and (**D**) show the 9 composite volumes for inlet and outlet reservoirs and how they depend on the angle and device geometry for negative and positive rocker angles. These sketches show the variables used in the derivation of an analytical shear stress value. From these sketches, the variables $h_{f,in}$ and $h_{f,out}$, defined as the height of the fluid along the right hand wall, are obtained. (**C**) shows an example of how V_{i22} and V_{i23} are calculated for θ<0.

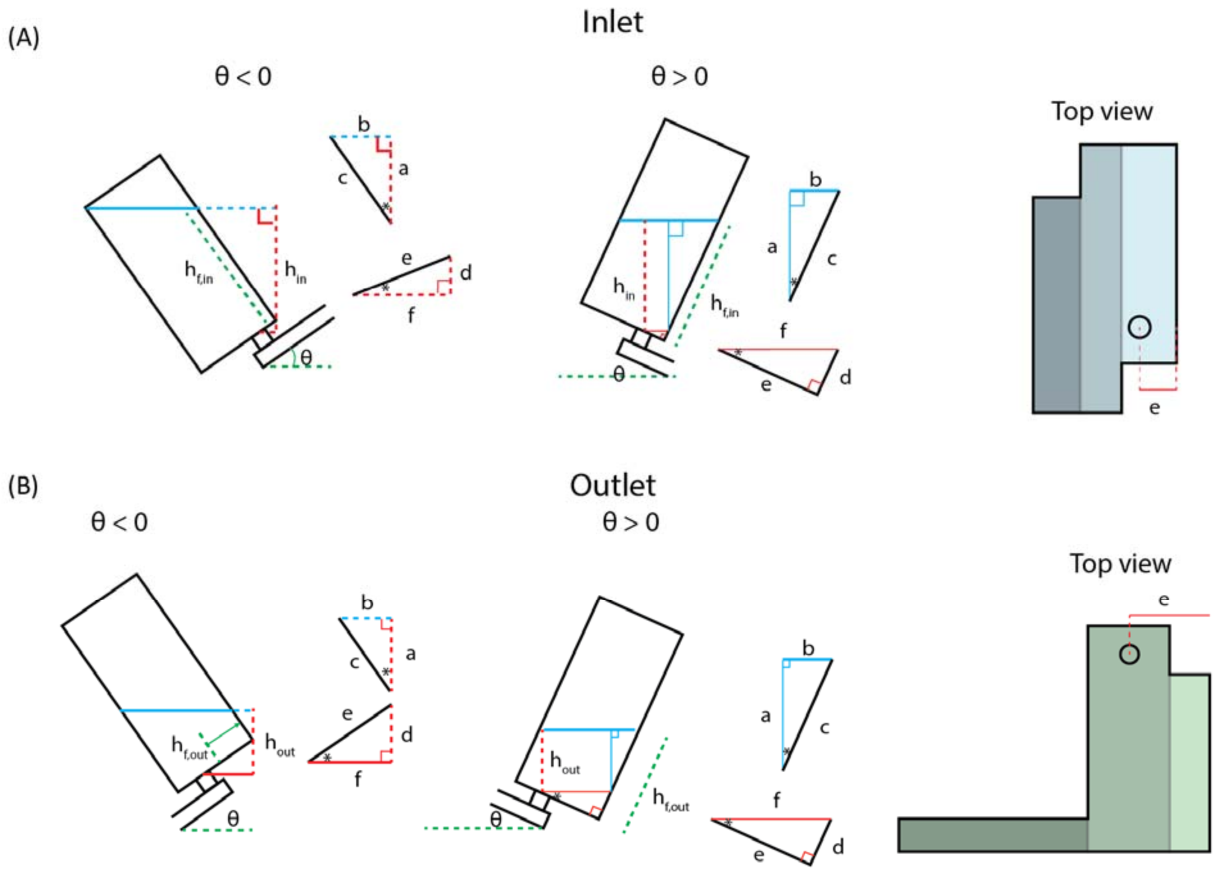


Fig. S3 Further geometric drawings for the derivation of an analytical expression for shear stress as a function of reservoir volume and rocker angle. These variable definitions are used to calculate the height of fluid above the reservoir ports, h_{in} and h_{out}, from the height of the fluid along the right-hand reservoir wall (see **Figure S1**). The height difference, *dh*, can be obtained from h_{in}, h_{out} and the height difference from the rocker itself (h_θ). In turn, *dh* yields flow which in turn is used to calculate shear stress.

1.5 dyn/cm^2

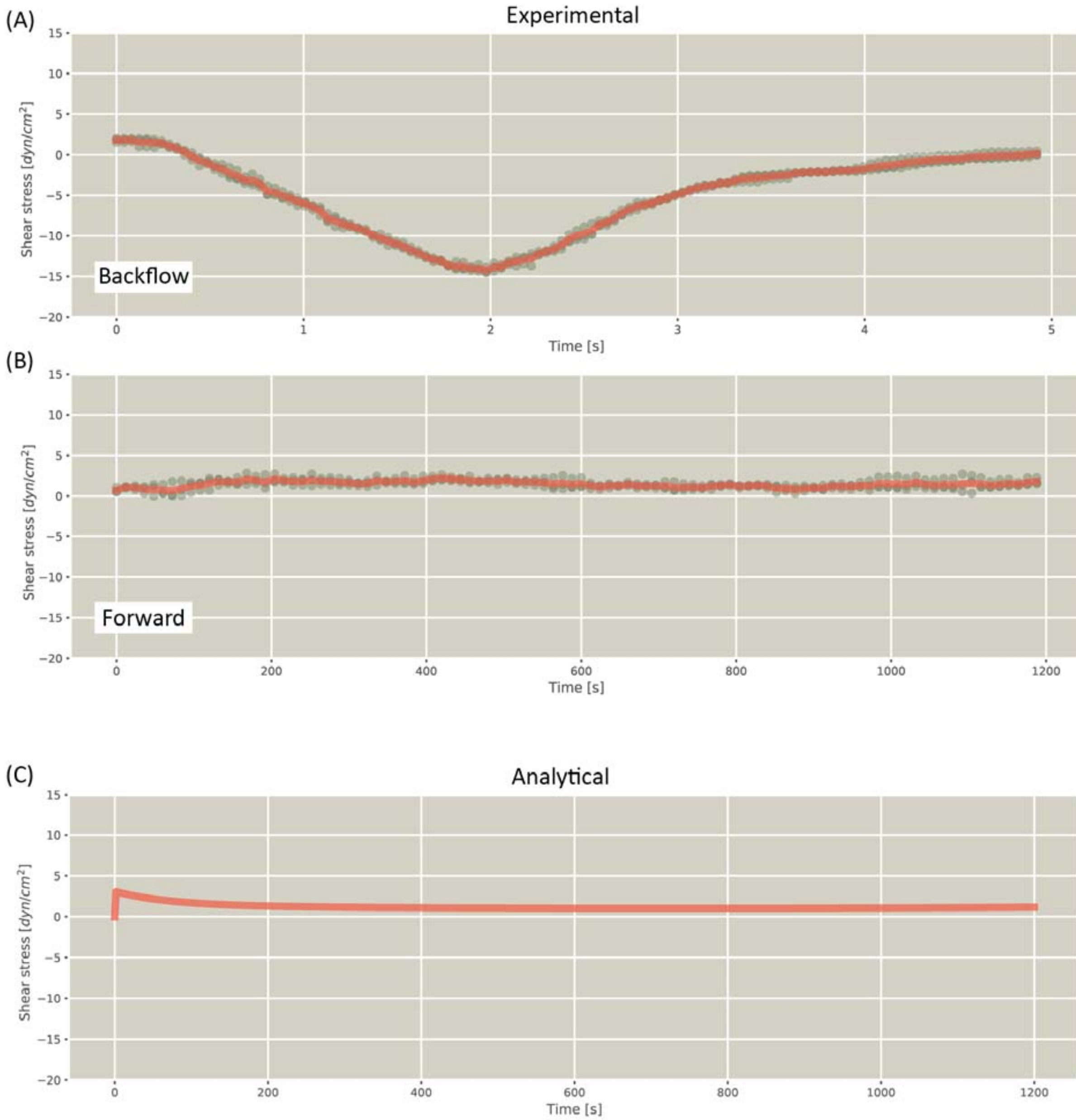


Fig. S4 Shear stress for the VitroFlo plate generated using 1.5 dynes / cm^2; 20 min forward cycle. (**A**) Experimental measurements for backflow cycle. Symbols show individual measurements, solid line shows averaged shear stress. (**B**) Experimental measurements for forward cycle. Symbols show individual measurements, solid line shows averaged shear stress. (**C**) Analytically derived shear stress values for forward cycle. The discrepancy between the analytically and the experimentally derived shear stress values originate in the surface interactions between fluid and VitroFlo plate, creating major meniscus effects which alter the geometry and volume of the fluid.

3 dyn/cm²

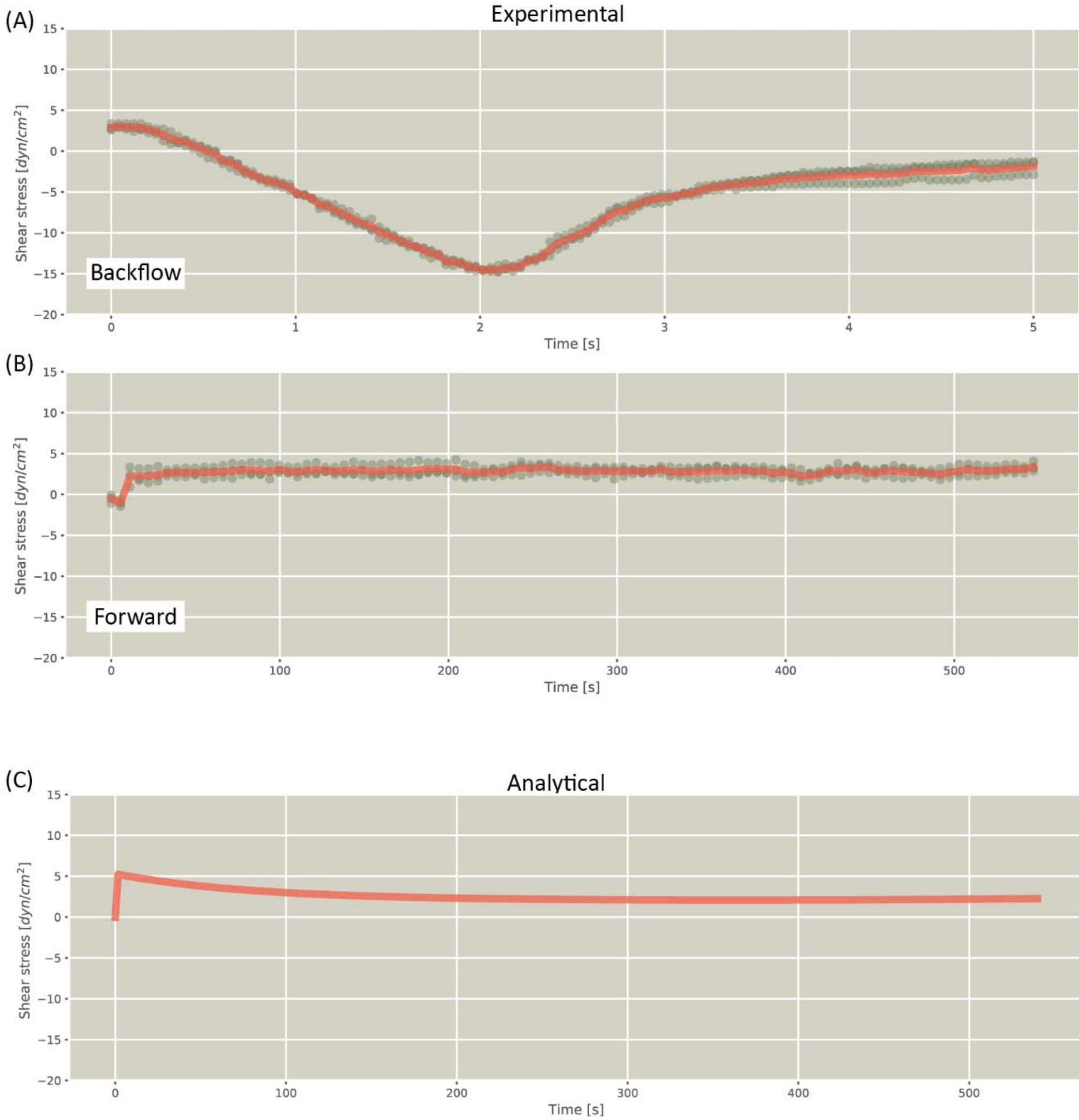


Fig. S5 Shear stress for VitroFlo plate generated using 3 dynes / cm^2; 9 min forward cycle. (**A**) Experimental measurements for backflow cycle. Symbols show individual measurements, solid line shows averaged shear stress. (**B**) Experimental measurements for forward cycle. Symbols show individual measurements, solid line shows averaged shear stress. (**C**) Analytically derived shear stress values for forward cycle. The discrepancy between the analytically and the experimentally derived shear stress values originate in the surface interactions between fluid and VitroFlo plate, creating major meniscus effects which alter the geometry and volume of the fluid.

5 dyn/cm^2

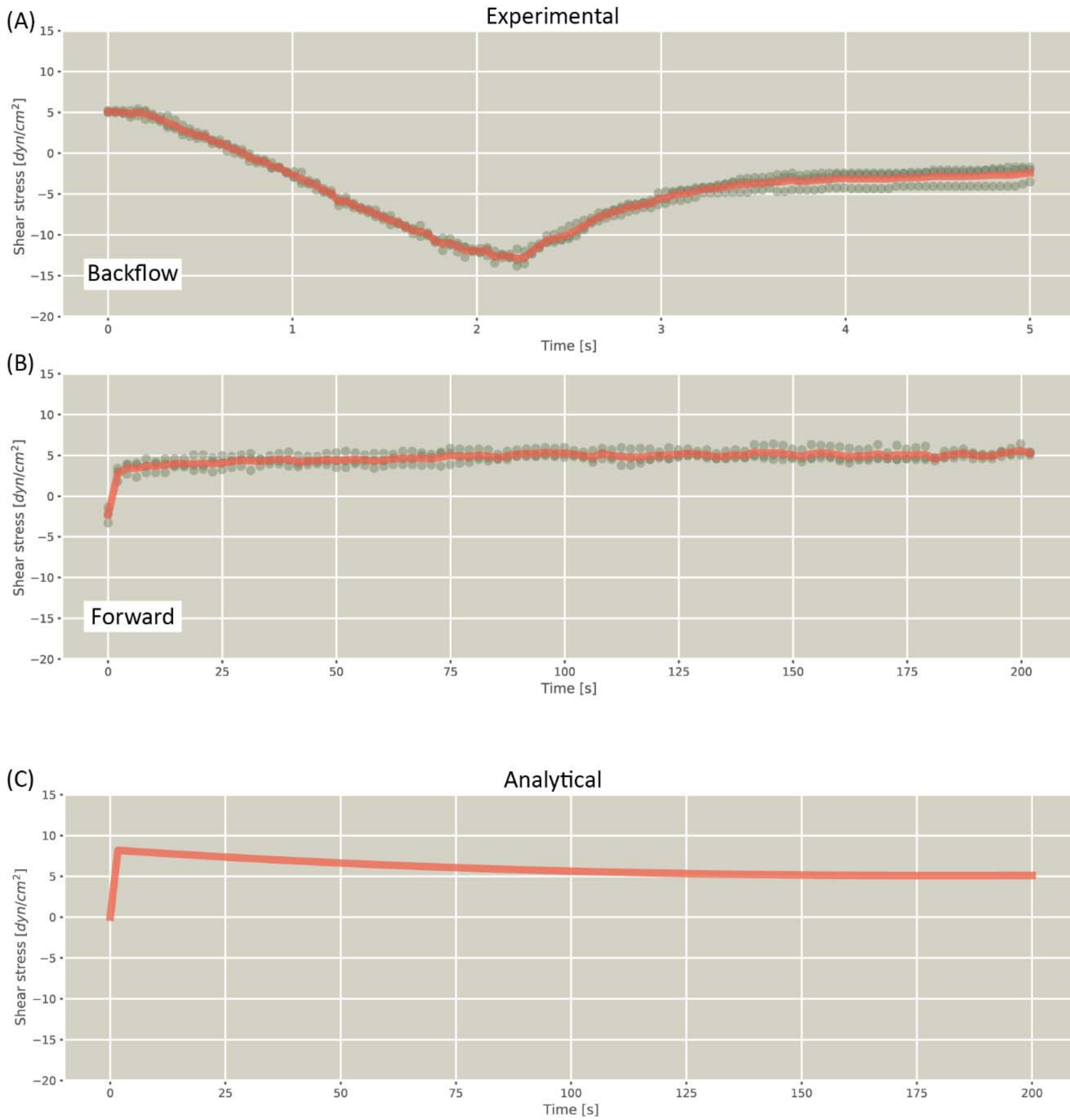


Fig. S6 Shear stress for VitroFlo plate generated using 5 dynes / cm^2; 3 min 20 s forward cycle. (**A**) Experimental measurements for backflow cycle. Symbols show individual measurements, solid line shows averaged shear stress. (**B**) Experimental measurements for forward cycle. Symbols show individual measurements, solid line shows averaged shear stress. (**C**) Analytically derived shear stress values for forward cycle. The discrepancy between the analytically and the experimentally derived shear stress values originate in the surface interactions between fluid and VitroFlo plate, creating major meniscus effects which alter the geometry and volume of the fluid.

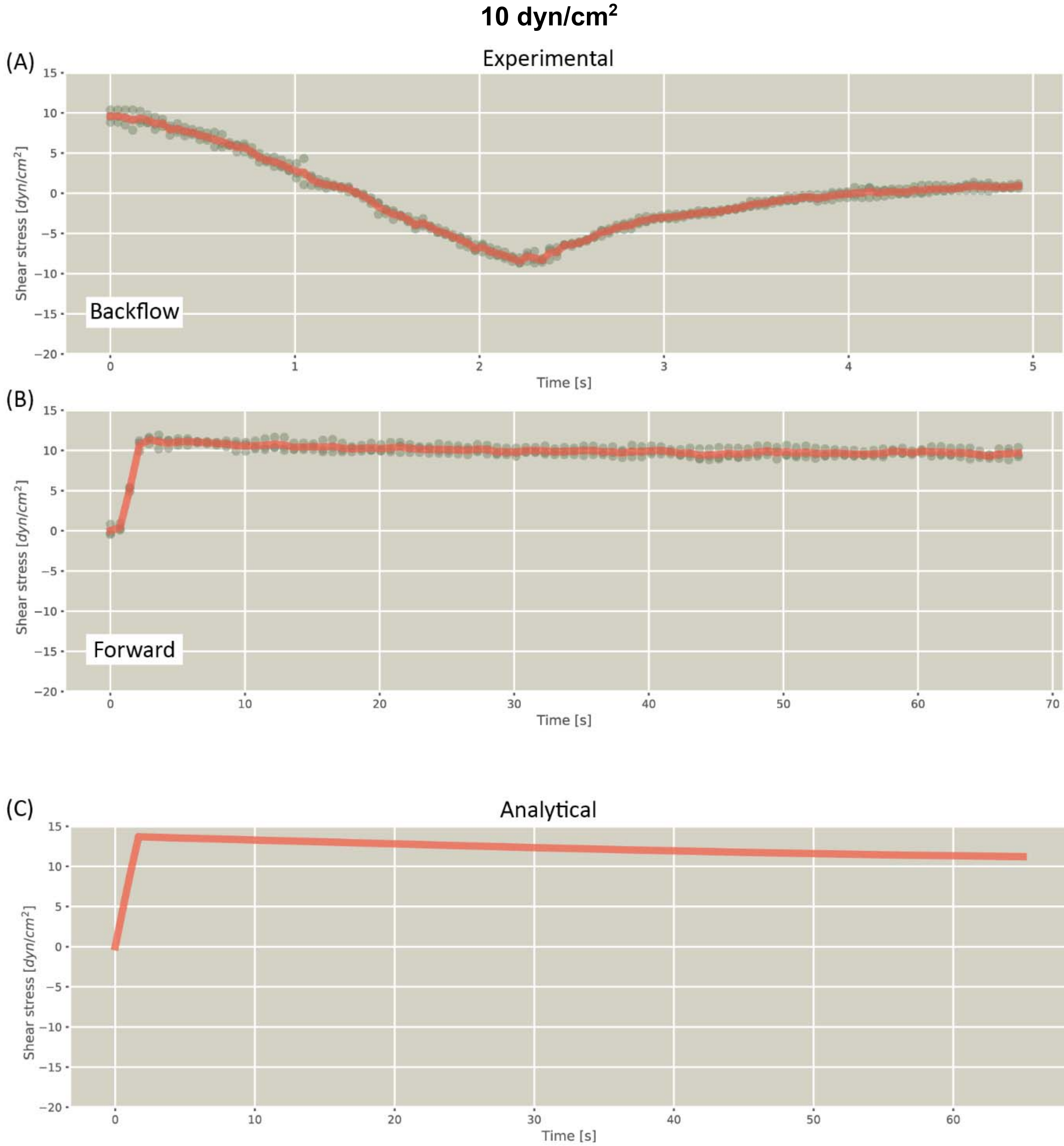


Fig. S7 Shear stress for VitroFlo plate generated using 10 dynes / cm^2; 65 s forward cycle. (**A**) Experimental measurements for backflow cycle. Symbols show individual measurements, solid line shows averaged shear stress. (**B**) Experimental measurements for forward cycle. Symbols show individual measurements, solid line shows averaged shear stress. (**C**) Analytically derived shear stress values for forward cycle. The discrepancy between the analytically and the experimentally derived shear stress values originate in the surface interactions between fluid and VitroFlo plate, creating major meniscus effects which alter the geometry and volume of the fluid.