Supplementary Material

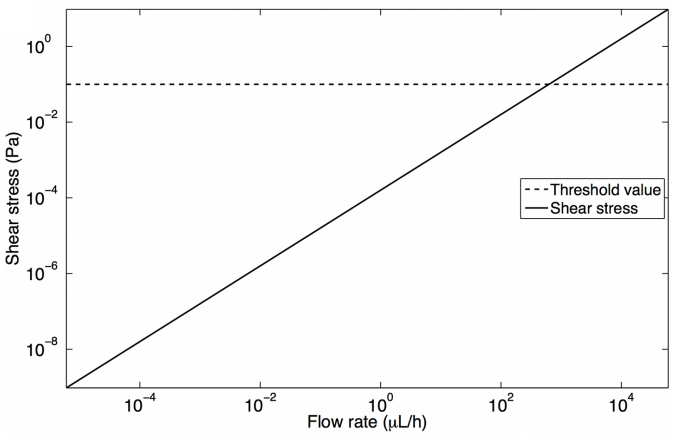

Fig. S 1 Steady state behaviour of the system as function of the flow rate for shear stress.

Computational model - Boundary conditions

In this work, we studied two different fluid dynamics cases, perfused and rest state; hence we had two sets of boundary conditions, they are summarized in Table 1. In the perfused state, fresh media is continuously pumped into the chamber. Cell metabolism generates a concentration gradient of oxygen and glucose from the medium bulk to the cell/medium interface, and an inward flux of EnF from the cells to the medium. At the top of the system an oxygen inward flux was considered due to the PDMS gas permeability.[17] In the rest condition there is no medium flow, thus at the inlet and outlet insulation conditions were used, while all the other conditions are the same.

Table S 1 Boundary conditions for the mass transport equations

Medium perfusion state			
Boundary	Oxygen	Glucose	EnF
Channel inlet	$N_{O_2} = \mathbf{v}c_{O_2}^{sat}$	$N_G = \mathbf{v}c_G^0$	$N_{EnF} = 0$
Channel outlet	$N_{O_2} = \mathbf{v}c_{O_2}$	$N_G = \mathbf{v}c_G$	$N_{EnF} = \mathbf{v}c_{EnF}$
Cell/medium inter.	$N_{O_2} = -R_{O_2}$	$N_G = -R_G$	$N_{EnF} = R_{EnF}$
Microchip top	$N_{O_2} = N_{O_2}^{ext}$	$N_G = 0$	$N_{EnF} = 0$
Others	$N_{O_2} = 0$	$N_G = 0$	$N_{EnF} = 0$

Medium rest state			
Boundary	Oxygen	Glucose	EnF
Channel inlet	$N_{O_2} = 0$	$N_G = 0$	$N_{EnF} = 0$
Channel outlet	$N_{O_2} = 0$	$N_G = 0$	$N_{EnF} = 0$
Cell/medium inter.	$N_{O_2} = -R_{O_2}$	$N_G = -R_G$	$N_{EnF} = R_{EnF}$
Microchip top	$N_{O_2} = N_{O_2}^{ext}$	$N_G = 0$	$N_{EnF} = 0$
Others	$N_{O_2} = 0$	$N_G = 0$	$N_{EnF} = 0$

Numerical model

The periodic configuration is characterized by the perfused and rest state. Both cases were described developing algorithms in COMSOL, which were embedded in a main MATLAB source (The Math- Works, Natick, MA) (Fig. S 2). This solution allowed to speed up the algorithm solving both cases consecutively. To this end a MATLAB/COMSOL interface was created. Starting

from the input data, the first time step ($t_{span} = \left[0, t_{fin}^{stat}\right]$) is solved (rest condition), afterwards COMSOL outcomes are stored in the MATLAB workspace; then the time span and the initial conditions are updated using the previous results in order to solve the perfused state. MATLAB algorithm iterates this process, using COMSOL functions, until reaches the final time. All model parameters used in the simulations are listed in Table S 2.

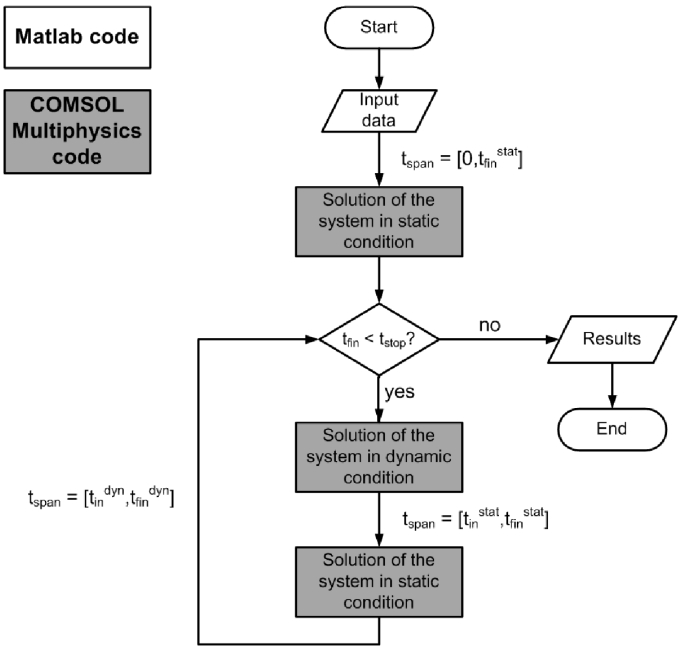

Fig. S 2 Scheme of the algorithm structure for solving the mathematical model of the microfluidic bioreactor.

Table S 2 Model parameters: their symbol, the respective values, and reference

Parameters	Symbol	Value	Units	Reference
Oxygen kinetic constant	V_{max}	2.06×10^{-16}	mol cell^{-1} s^{-1}	19
Oxygen kinetic constant	K_m	5.0×10^{-3}	mol m^{-3}	19
Glucose kinetic constant	k_1	9.75×10^{-18}	m^3 cell^{-1} s^{-1}	Experimental value
Glucose kinetic constant	k_2	2.97×10^{-18}	mol cell^{-1} s^{-1}	Experimental value
EGF specific production rate	q_{EGF}	1.67×10^{-22}	mol cell^{-1} s^{-1}	20
Cell doubling time	τ_d	16	h	Experimental value
Cell growth kinetic constant	μ	1.203×10^{-5}	s^{-1}	Experimental value
Medium density	ρ_{med}	1002.5	kg/m^3	Experimental value
Medium viscosity	μ_{med}	7.7×10^{-4}	Pa s	23
Oxygen diffusion coefficient in medium	D_{O_2}	3.29×10^{-9}	m^2/s	24
Glucose diffusion coefficient in medium	D_G	9×10^{-10}	m^2/s	25
EGF diffusion coefficient in medium	D_{EGF}	2×10^{-10}	m^2/s	26
PDMS permeability to oxygen	P_m	2.84×10^{-13}	mol m m^{-2} s^{-1} Pa^{-1}	18
Atmospheric oxygen partial pressure in incubator	p_{O_2}	1.9×10^4	Pa	27
Henry's coefficient for oxygen	k_{O_2}	1.04×10^5	Pa m^3 mol^{-1}	28
Glucose concentration in fresh medium	c_G^0	5.28	mol/m^3	Experimental value
PDMS layer thickness	σ	5×10^{-3}	m	Experimental value
Cell density at confluence	ρ_{cell}^{max}	7.58×10^8	cell/m^2	Experimental value
Shear stress threshold	τ_{max}	0.1	Pa	29

Computational model validation

The mathematical model of the microfluidic cell culture system was validated by comparison with experimental data using the continuous protocol; in particular we analyzed the cell growth and the cell metabolism. The total cell number in the microfluidic channel was determined by means of image analysis; it was compared with the model outcomes (Fig. S 3a). Theoretical data are able to describe the experimental results, however it underestimates these values for a long time values. This is probably due to the cell ability to growth forming a partial second layer, this phenomenon was not taken into account by the model. The cell metabolism was evaluated measuring the glucose consumption rate. To this end, we compared the glucose concentration at the outlet of the system (Fig. S 3b), experimental data are well described by our model.

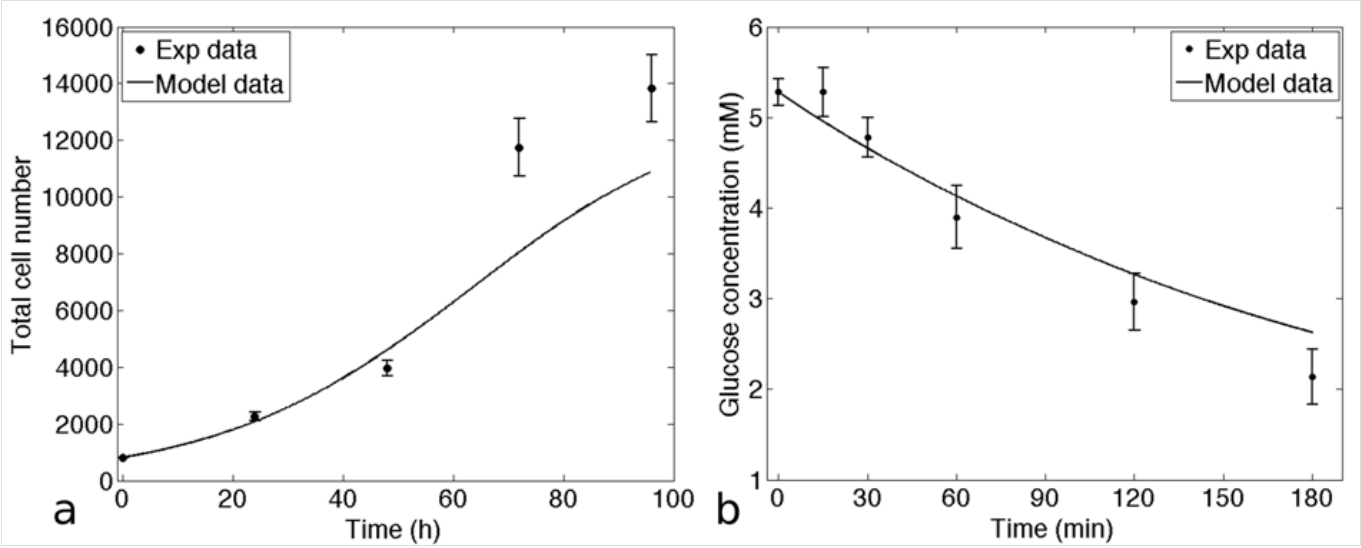

Fig. S 3 Comparison between experimental data and computational model in C1 condition for both cell growth and dowstream glucose detection.

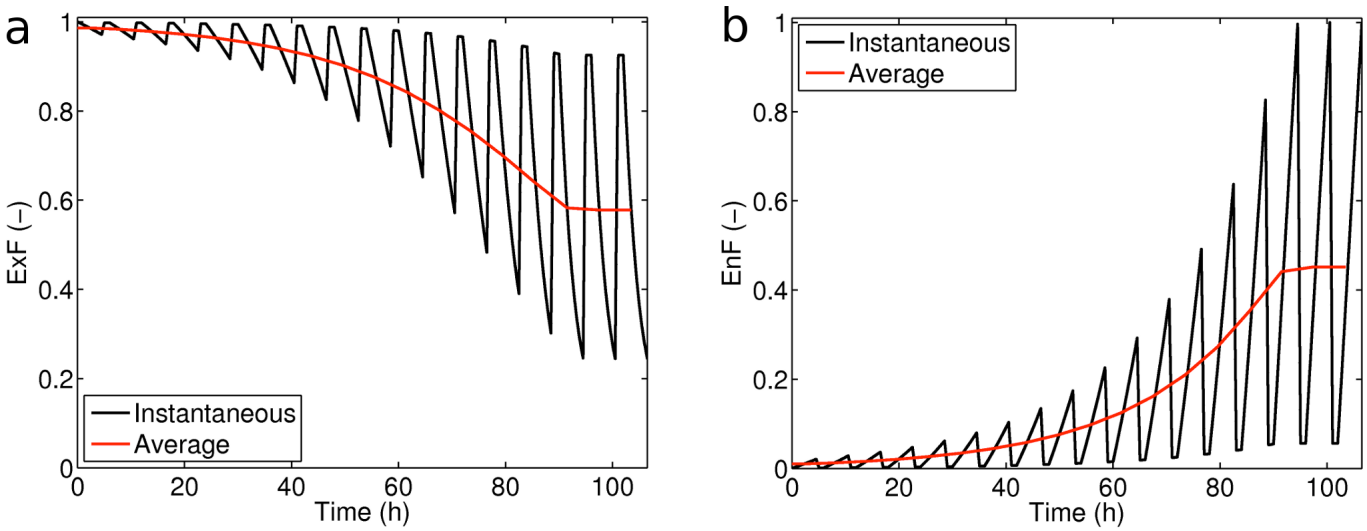

Fig. S 4 Temporal evolution of a microfluidic cell culture growth system with a cyclic flow rate delivery. (a) and (b) show the glucose and EnF concentration evolution at the cell surface, the red line represents the average value calculated at each cycle.

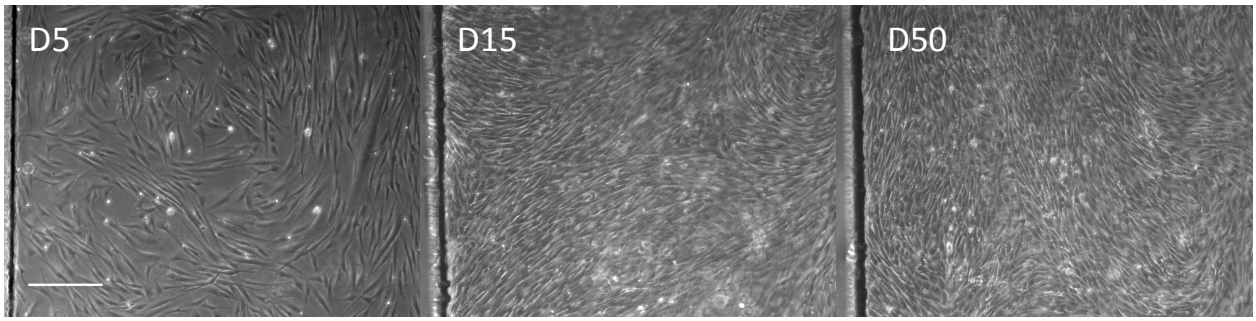

Fig. S 5 HFF cells cultivated with periodic strategy P2' without passaging. Channel wall on the left-side of each image. Cells reached confluence within the first week and progressively packed reducing the projection area. Seeding density is reported in material and methods. Figures report cell within the channel ad day 5, 25 and 50. Scalebar 100 μm.

Quantification of colony size and marker expression for different perfusion strategies

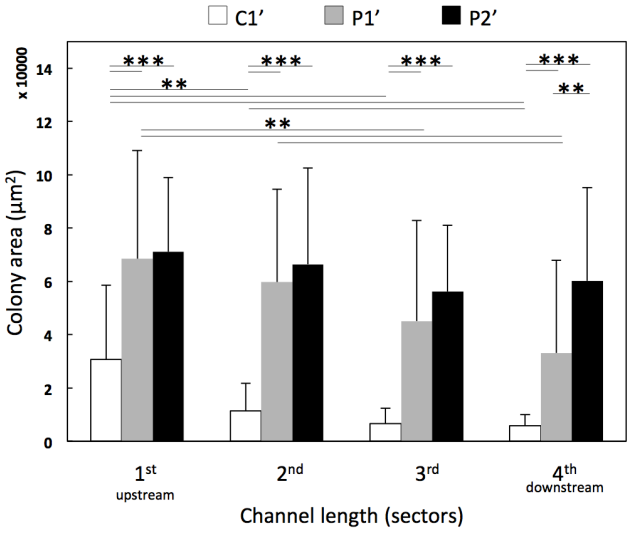

Fig. S 6 Quantification of mESC colony size for C1', P1', P2' strategies. Each channel was divided in four equivalent sectors, starting from the upstream region (first sector). mESC colonies had an homogeneous distribution along the channel. Both in C1' and P1' mESC colonies size decreased towards downstream region (forth sector). In C1', upstream colonies had reduced size compared to both perdioc flows. Data are reported as mean value and standard deviation. n=25; ANOVA analysis *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

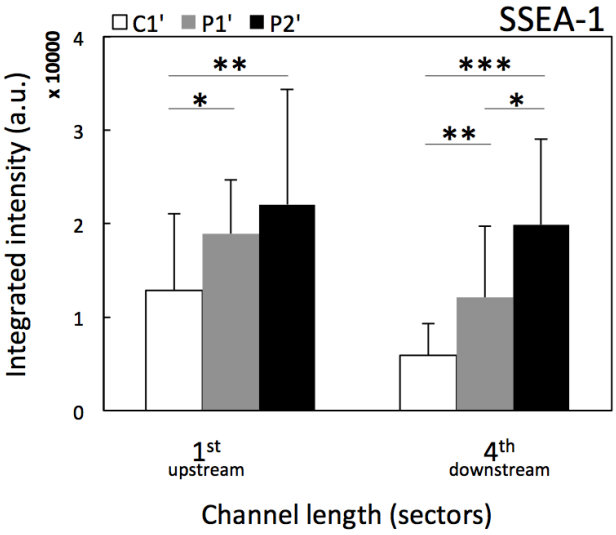

Fig. S 7 Quantification of SSEA-1 expression within colonies for C1', P1', P2' strategies. Mean intensity within each colony was multiplied by the colony size as integrated intensity in ImageJ software analysis. In P2' strategy, colonies show increased levels of SSEA-1 pluripotency marker both upstream and downstream. Data are reported as mean value and standard deviation. n=20; ANOVA analysis *: p<0.05; **: p<0.01; ***: p<0.001.

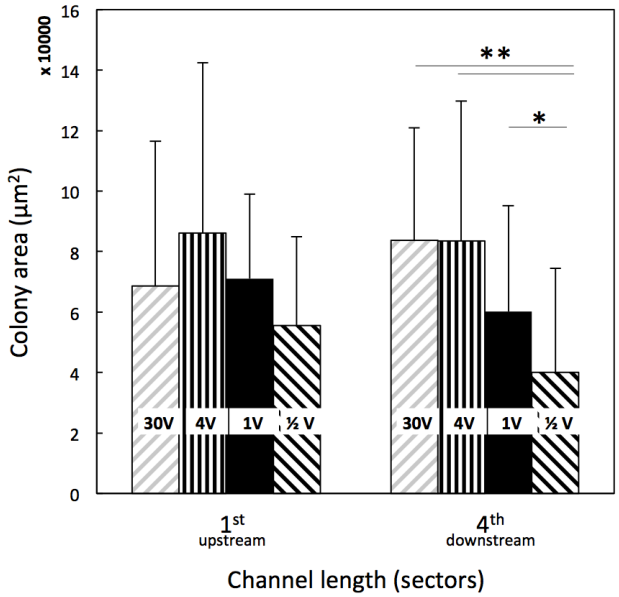

Fig. S 8 Quantification of mESC colony size for C3', P4', P2', P5' strategies. Increasing the flow rate mESC growth can be rescued. An increase of 30- and 4-fold of the perfused volume in a cycle has been applied compared to C1' and P1' respectively. Reducing the perfused volume of P1' (1/2V), colony size was reduced. Data are reported as mean value and standard deviation. n=25; ANOVA analysis *: p<0.05; **: p<0.01.

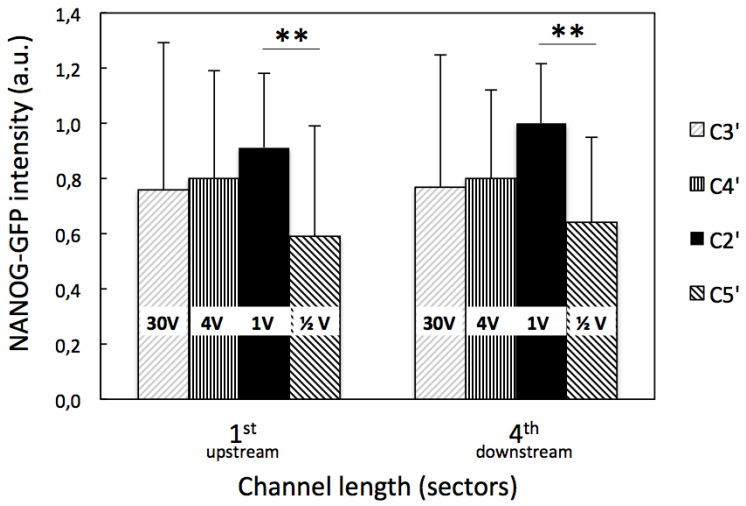

Fig. S 9 Quantification of NANOG-GFP fluorescence intensity per single colony for C3', P4', P2', P5', strategies. Increasing the flow rate, mES colonies had a rescue in NANOG expression. An increase of 30- and 4-fold of the perfused volume in a cycle has been applied compared to C1' and P1' respectively. NANOG-GFP reporter intensity was significantly reduced when the perfused volume of P1' decreased (1/2V). Data are reported as mean value and standard deviation. n=20; ANOVA analysis **: $p<0.001$.