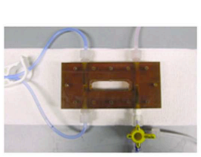
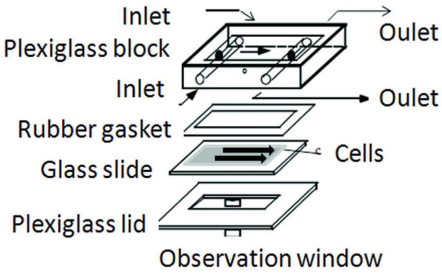
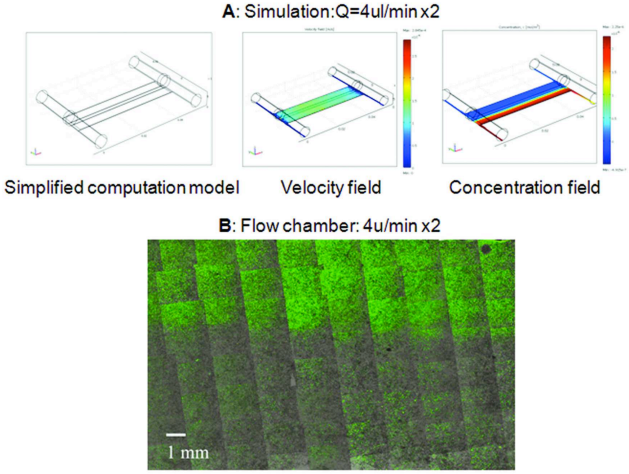


Flow chamber

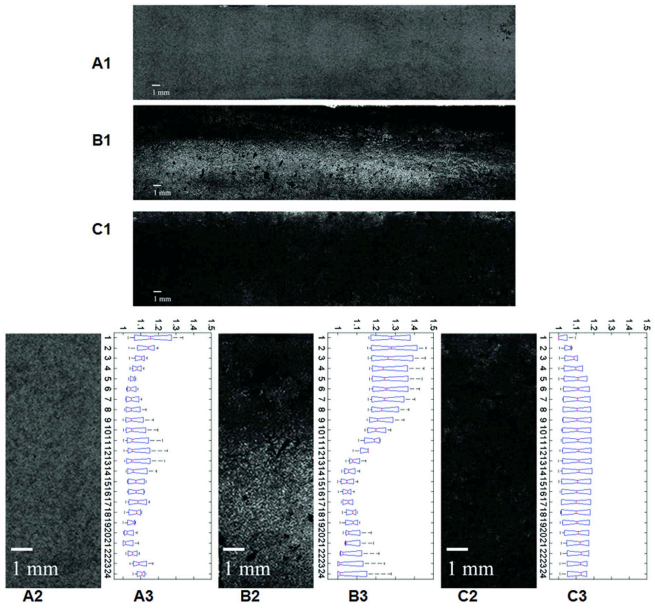


Supplemental Fig. 1 Flow chamber. The flow chamber consisted of a 1mm thick borosilicate glass plate glued into the bottom of a uniformly deep groove milled into a Plexiglas block, and another glass slide with pre-seeded cells. The two plates were held together with screws and a Plexiglas lid, separated by a uniform thickness rectangular silastic spacer (McMaster). As a result, a flow chamber (5.2 cm long x 1.2cm wide x 154μm high) was created. Flow inlets and outlets were connected to cylindrical reservoirs in the Plexiglas block. A 2mm thick borosilicate glass blocked each of the cylindrical reservoirs in the middle, and separated the flows to and from the two ports. Plastic tubing (high-temperature silicone rubber soft tubing, 1.59mm ID, 3.18mm OD, McMaster) connected the inlet port to one-direction syringe pump (Harvard) and the outlet port to the waste collecting bottles.

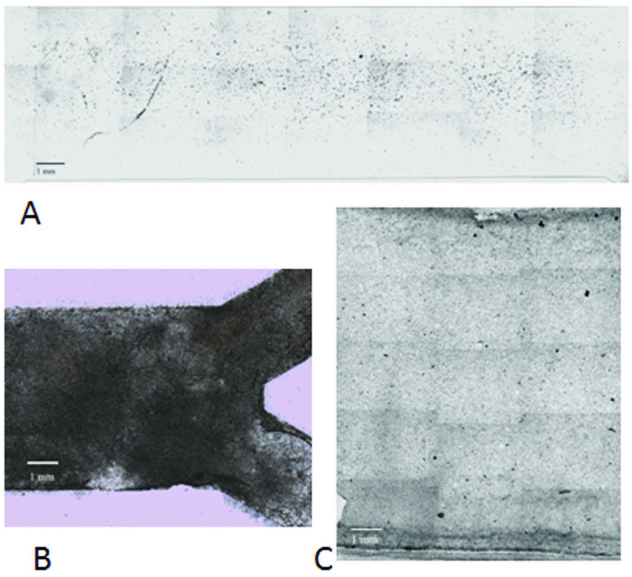
Flow Chamber: Cell culture area 624 mm²		
L	W	H
52 mm	12 mm	0.154 mm
Flow Rate: Q	Shear Stress: τ	Reynolds: Re
8 µl/min x 2	5.6 mPa	0.044
4 µl/min x 2	2.8 mPa	0.022



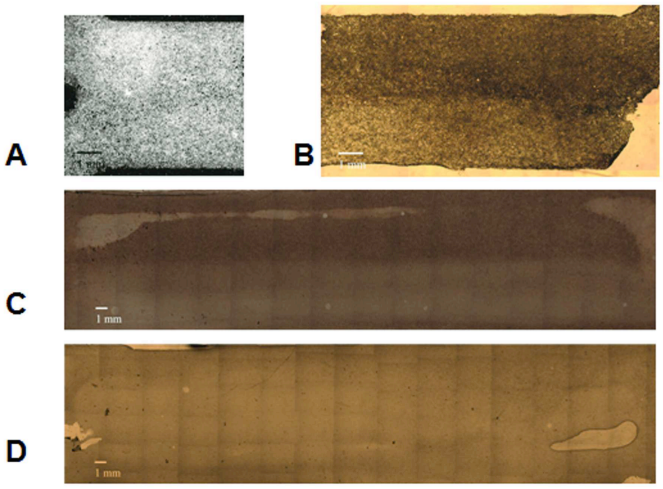
Supplemental Fig. 2: Characterization of flow chamber. A: Simplified flow models, the steady-state velocity and concentration profiles are shown for the flow channel at flow rate of 4µl/min x2. Data were calculated for the diffusion coefficient DDox=3.93x10-6 cm2/s, fluid viscosity v=10-3Pa*s, fluid density ρ=103kg/m3, and inlet concentrations CDox=1ng/ml= 2.25x10-12mol/ml and CDox=0ng/ml= 0. B: Separation of Calcein stain in the flow channel at the flow rates of 4µl/min x 2. Data are for the diffusion coefficient of Calcein DCalcein=2.6*10-6 cm2/s, and inlet concentrations of Calcein C+=2µM and C-=0, diffusion coefficient of Flow direction is left to right. Original magnification: 100X. Scale bar: 1mm.



Supplemental Fig. 3: Dox dependent osteogenic differentiation in flow chamber. C9 cells were cultured in osteogenic differentiation medium in a perfused flow chamber, at a flow rate of 4µl/min x2, for 3 weeks. Images show von Kossa staining of calcium deposition for selected conditions: (A1, A2) Perfusion medium with Dox 1ng/ml (top) / 1ng/ml (bottom); (B1, B2) Perfusion medium: Dox 0ng/ml (top) / 1ng/ml (bottom); (C1, C2) Perfusion medium: Dox 0ng/ml (top) / 0ng/ml (bottom). (A3, B3, C3) Intensity of calcium staining, obtained by pooling repeated experiments (n=4 per condition). The staining of the deposited calcium is expressed at the ratio of the normalized sum of segmented (~0.25mm) image intensity and the minimum intensity along the y axis (the width) of the chamber. Data were processed using Matlab R2007 program. Flow direction: left to right. Scale bar: 1mm



Supplemental Fig. 4: The effect of flow rate to osteogenic differentiation. A: von Kossa staining of the fluidic channel, at flow rate of 5µl/min x2. Perfusion medium: Dox 0ng/ml (top) / 1ng/ml (bottom); B: von Kossa staining of the fluidic channel, at flow rate of 0.5µl/min x2. Perfusion medium: Dox 0ng/ml (top) / 0ng/ml (bottom); C: von Kossa staining fragment of the fluidic chamber, at flow rate of 8µl/min x2. Perfusion medium: Dox 0ng/ml (top) / 0ng/ml (bottom).



Supplemental Fig. 5: Cell distribution and BSP staining. A: Phase contrast image of cell distribution in fluidic channel after 3 weeks osteogenic differentiation at flow rate of 1µl/min x2. Perfusion medium: Dox 0ng/ml (top) / 1ng/ml (bottom). B: BSP staining of the cells after 3 weeks patterned osteogenic differentiation in fluidic channel at flow rate of 1µl/min x2. Perfusion medium: Dox 0ng/ml (top) / 1ng/ml (bottom). BSP staining of the cells after 3 weeks patterned osteogenic differentiation in fluidic chamber at flow rate of 4µl/ml x2. C: Perfusion medium: Dox 0ng/ml (top) / 1ng/ml (bottom); D: Perfusion medium: Dox 1ng/ml (top) / 1ng/ml (bottom); Flow direction: left to right.