Electronic Supplementary Material (ESI) for Integrative Biology

Supporting Information for:

Modulation of alignment and differentiation of skeletal myoblasts by biomimetic materials†

Ilaria E. Palamà[a,b*], Addolorata M. L. Coluccia[c], Giuseppe Gigli[a,d,e] and Mathis Riehle[f]

[a]NNL, Institute of Nanoscience CNR, Via Arnesano 73100 Lecce, ITALY
[b]Scuola Superiore ISUFI, University of Salento, Via Arnesano 73100 Lecce, ITALY
[c]Hematology and Clinical Proteomics Unit, "Vito Fazzi" Hospital, University of Salento, Piazzale Muratore, 73100 Lecce, ITALY
[d]Departement of Mathematics and Physics,University of Salento, Via Monteroni 73100 Lecce, ITALY
[e]Center fo Biomolecular Nanotechnologies (CNB) of Italian Institute of Technology (IIT), Via Barsanti 73010 Arnesano (Lecce), ITALY
[f]Centre for Cell Engineering, Institute of Molecular, Cell and Systems-Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Joseph Black Building, G12 8QQ, Glasgow, UK

**Corresponding authors. e-mail:* ilaria.palama@unisalento.it

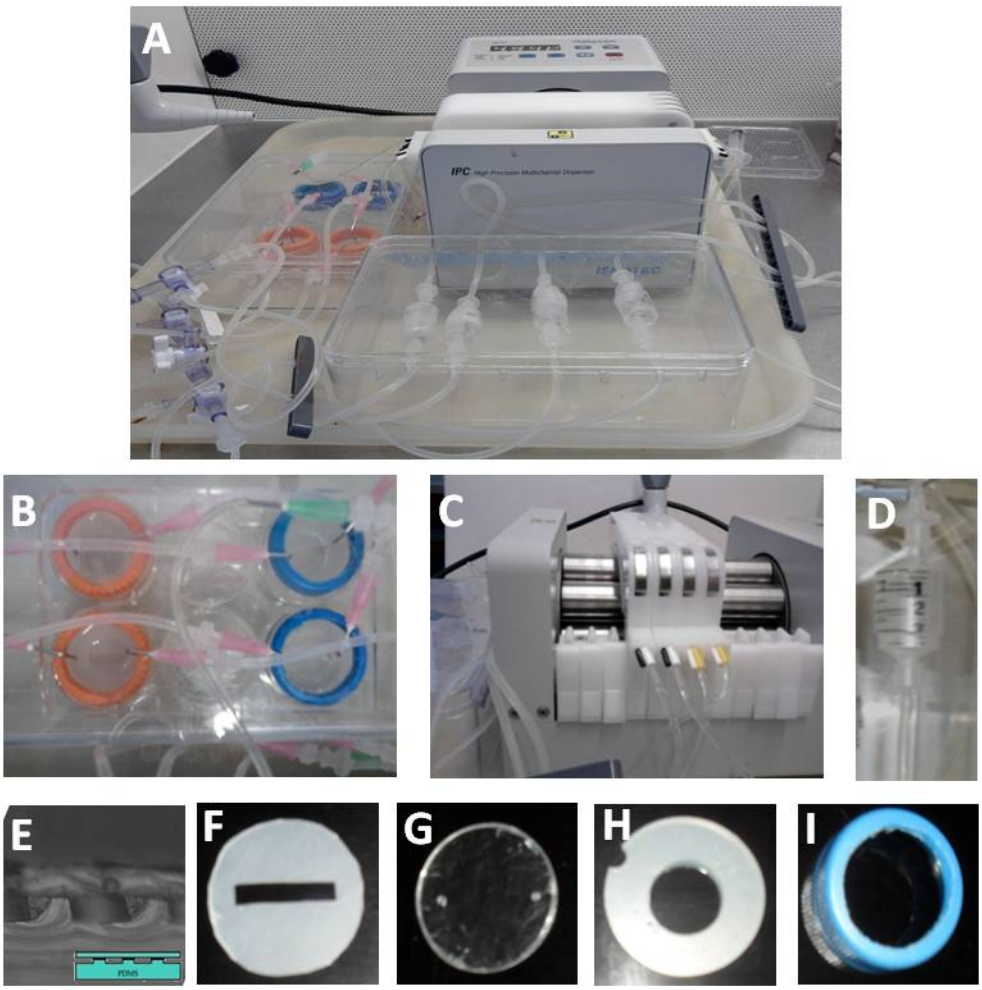


Figure S1. The bioreactor system (A) includes mini perfusion chambers (B), peristaltic pump (C) and medium reservoir (D). The mini perfusion chambers (B) were constructed in the 6-well plate. A sandwich of the following parts was assembled inside the wells to form the perfusion chambers: at the bottom a piece of rectangular PDMS substrate (E), followed by a circular silicon spacer (F), a circular transparent polystyrene plate with two small holes (G), a circular silicone sealing (H). The PDMS substrate, the silicon spacer and the polystyrene plate together formed the mini perfusion chamber that was clamped by the plastic o-ring (I). Two needles were inserted into the perfusion chamber and were used as inlet and outlet. Each perfusion chamber was connected by silicon tubes to a channel of the peristaltic pump and to a medium reservoir.

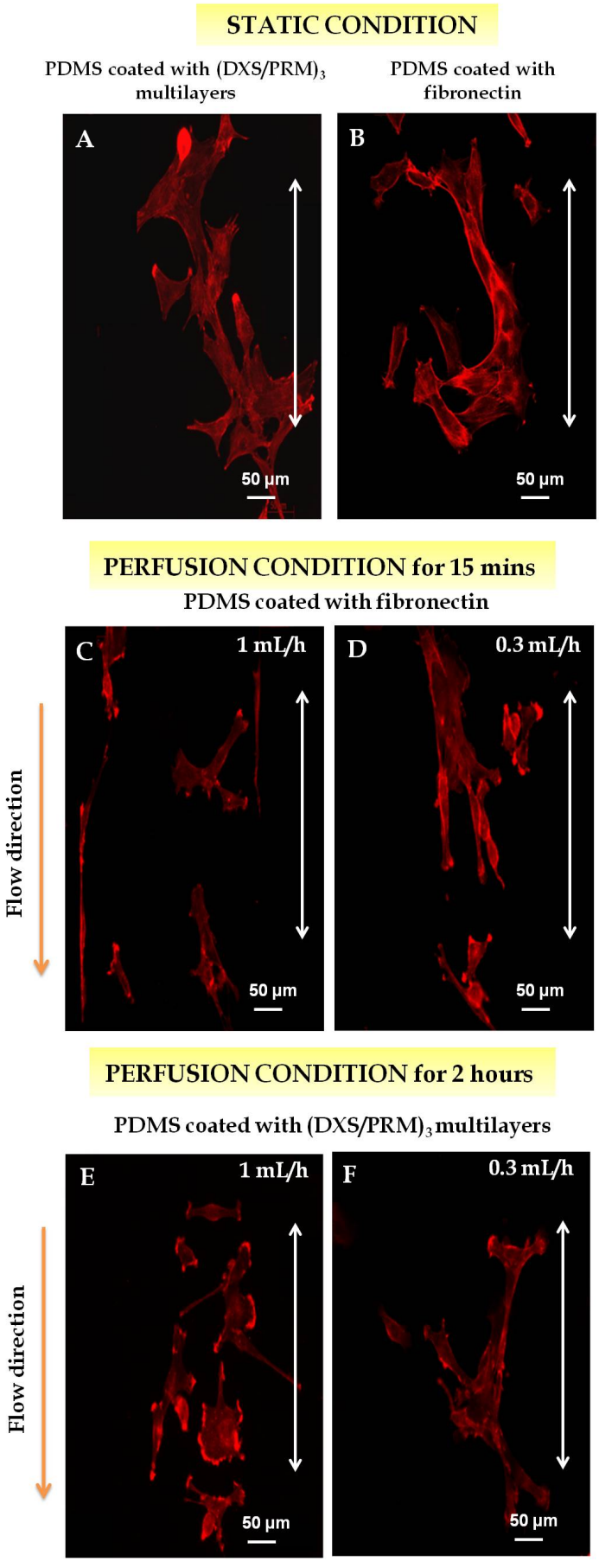
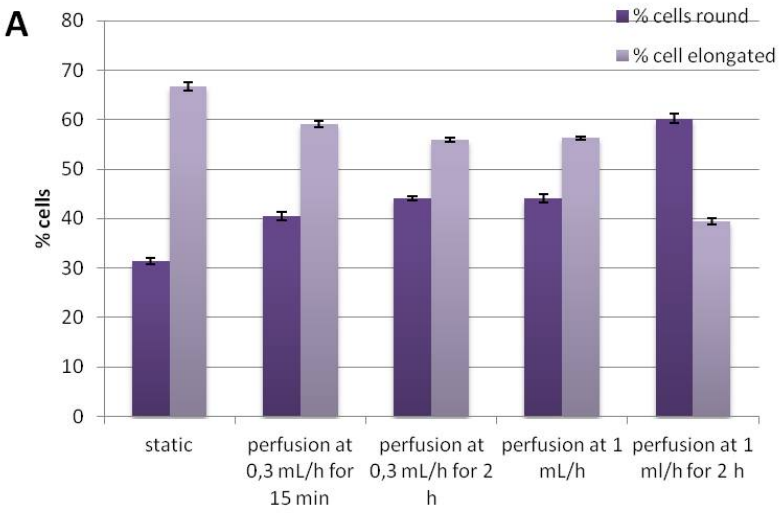
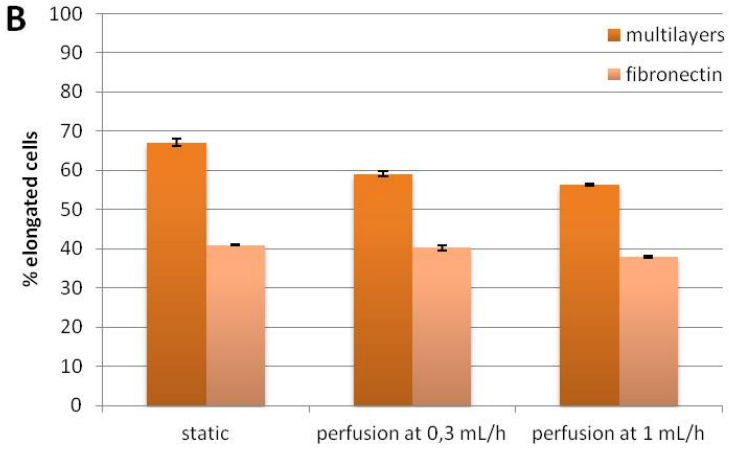


Figure S2. Fluorescent images of C2C12 cells seeded on substrates coated with fibronectin or $(DXS/PRM)_3$ multilayers under static (A,B) or perfusion condition with varying flow rates, from 1 mL/h to 0.3 mL/h for 15 min for PDMS coated with fibronectin (C,D) and 2 hours (E,F) for PDMS coated with PEMs and stained with fluorescent phalloidin (red). Nuclei stained with DAPI (blue). The white arrows indicate the directions of the patterns, while the pink arrows indicate the directions of the flow. *Scale bar*: 50 µm





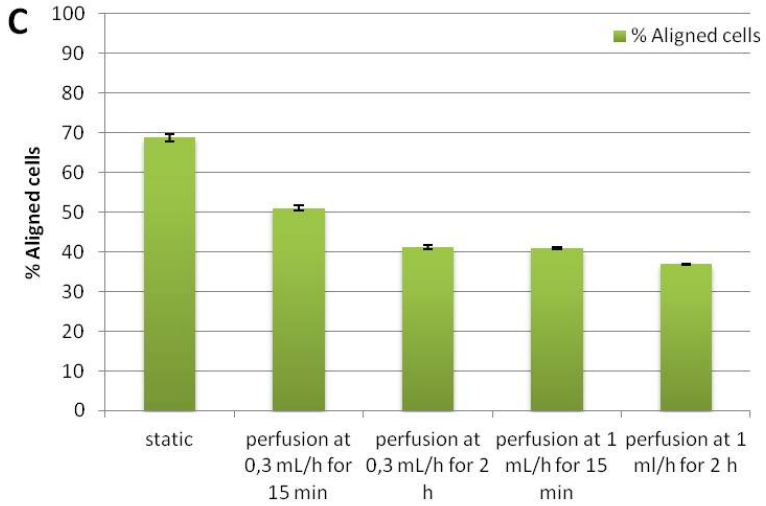


Figure S3. Evaluation of C2C12 cell morphology on substrates coated with fibronectin or $(DXS/PRM)_3$ multilayers under static or perfusion condition with varying flow rates, from 0.3 mL/h

to 10 mL/h for 15 min or 2 hours: A) Percentage of elongated and round cells when cultured on polyelectrolyte multilayers in static condition and in bioreactor at 0.3 mL/h or 1 mL/h fluid rate for 2 hours; (B) Percentage of elongated cells when cultured on polyelectrolyte multilayers or fibronectin in static condition and in bioreactor at different fluid rate for 15 min.; (C) Percentage cells that had orientation angle of 0° when cultured on polyelectrolyte multilayers in static condition and in bioreactor at 0.3 mL/h or 1 mL/h fluid rate for 2 hours. The results are expressed as mean ± standard deviations and are representative of five measurements of each fluorescent image (*t*-Student test, $P < 0.05$); cell numbers between 140 and 280.

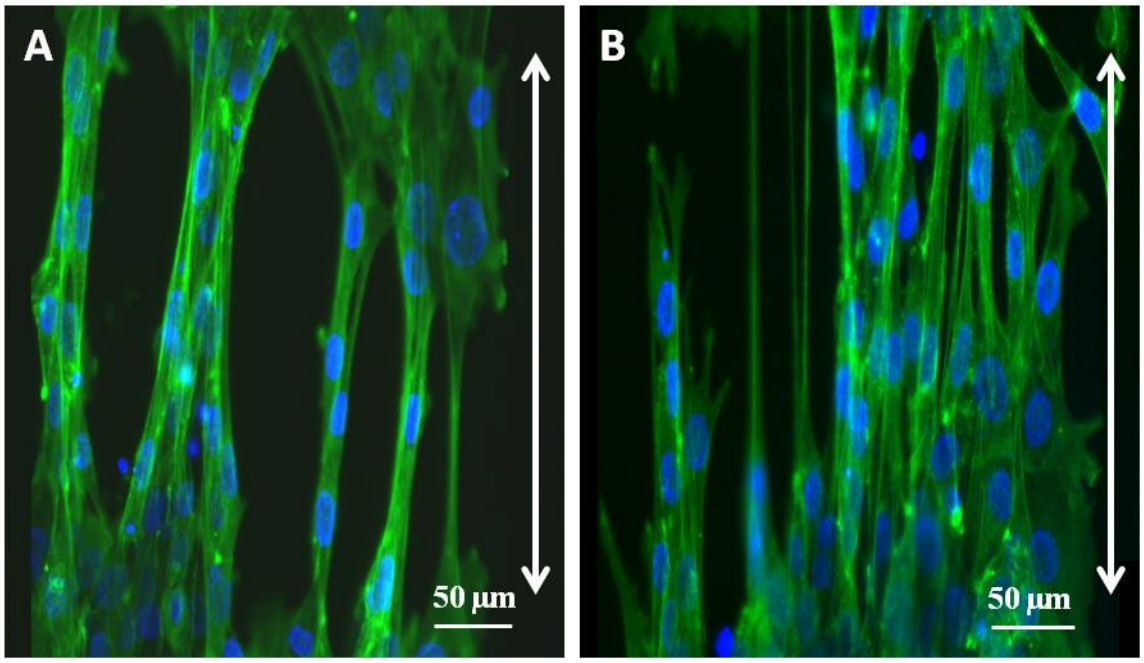


Figure S4. Fluorescent images of myoblasts grow in static condition for 7 days, even seeded at low cell density on control PDMS surfaces (without PEM's coating, A) and on PDMS substrates with stiffness modulation and $(DXS/PRM)_3$ multilayers coating (B) and stained with α-actinin (green). Nuclei stained with DAPI (blue). Myoblasts differentiation is carried out in growth medium, any switching in differentiative medium. The white arrows indicate the directions of the patterns. *Scale bar*: 50 µm