Stoichiometric control of live cell mixing to enable fluidically-encoded co-culture models in perfused microbioreactor arrays

Occhetta P<sup>a,b</sup>, Glass N<sup>a</sup>, Otte E<sup>a,d</sup>, Rasponi M<sup>b</sup>, Cooper-White J<sup>a,c,d,*</sup>

<sup>1</sup>Australian Institute of Bioengineering and Nanotechnology, The University of Queensland, Brisbane, QLD, Australia
<sup>2</sup>Department of Electronics, Information and Bioengineering, Politecnico di Milano, Milano, Italy
<sup>3</sup>School of Chemical Engineering, The University of Queensland, Australia 4072
<sup>4</sup>Biomedical Manufacturing, Manufacturing Flagship, CSIRO, Clayton, Victoria, Australia, 3169
* - corresponding author (j.cooperwhite@uq.edu.au)

Supplementary information

Fig SI1 An <i>ad hoc</i> device layout was conceived for preliminary mixing validations, consisting of (i) a three level serial dilution generator integrated with herringbone units for chaotic mixing (blue features) and (ii) a culture area comprising five parallel units. Dilutions of cells were generated from two main inlets (A1-A2) and delivered to downstream culture units. Each culture unit consisted of a rectangular culture chamber (w3000 x l6000 x h100 μm) and was combined with a lateral seeding channel.
Fig. SI2 Establishment of programmed hMSCs-SAOS2 osteogenic co-culture models within the microfluidic platform. Two different models were generated: a reverse gradient of hMSCs-SAOS2 (a) and a low concentration gradient of SAOS2 on a preseeded monolayer of hMSCs (b). The achievement of predicted mixing ratios is qualitatively visualized by DiD-staining of hMSC.

(a) hMSC/SAOS2 reverse gradient

(b) SAOS2 low concentration gradient

Fig. SI3 hMSCs-SAOS2 perfusion co-culture model: impact of SAOS2 concentration on alkaline phosphatase expression and cell proliferation. Fluorescence images showing ELF97 expressions for each considered hMSCs/SAOS2 co-culture ratios (8:0, 8:1, 8:2, 8:3 and 8:4), after 7 days under either maintenance or osteogenic medium slow perfusion (2µl/hr each column). Shown results correspond to two technical repeats of the experiments and five representative chambers are shown for each bioreactor unit (scale bar=1mm).
Fig. SI4 hMSCs-SAOS2 static co-culture controls: impact of SAOS2 concentration on alkaline phosphatase expression under macroscale static culture conditions.