

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

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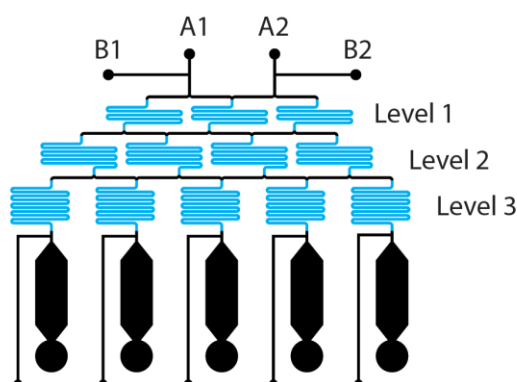
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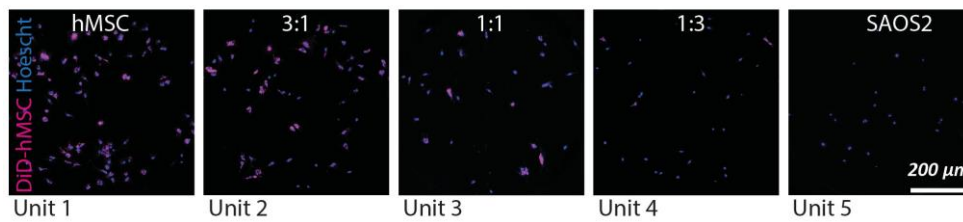
### Supplementary information

#### Preliminary validation device layout

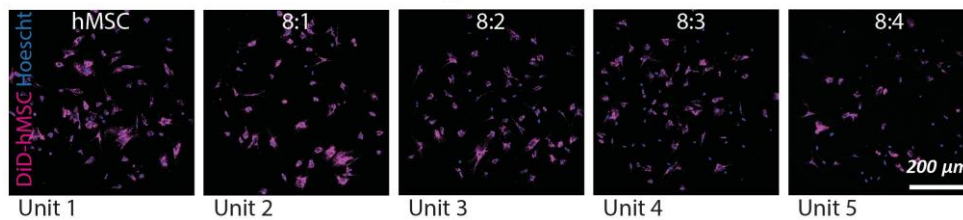


**Fig S11** An *ad hoc* device layout was conceived for preliminary mixing validations, consisting of (i) a three level serial dilution generator integrated with herringbone units for chaotic mixing (blue features) and (ii) a culture area comprising five parallel units. Dilutions of cells were generated 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  $\mu\text{m}$ ) and was combined with a lateral seeding channel.

(a) hMSC/SAOS2 reverse gradient

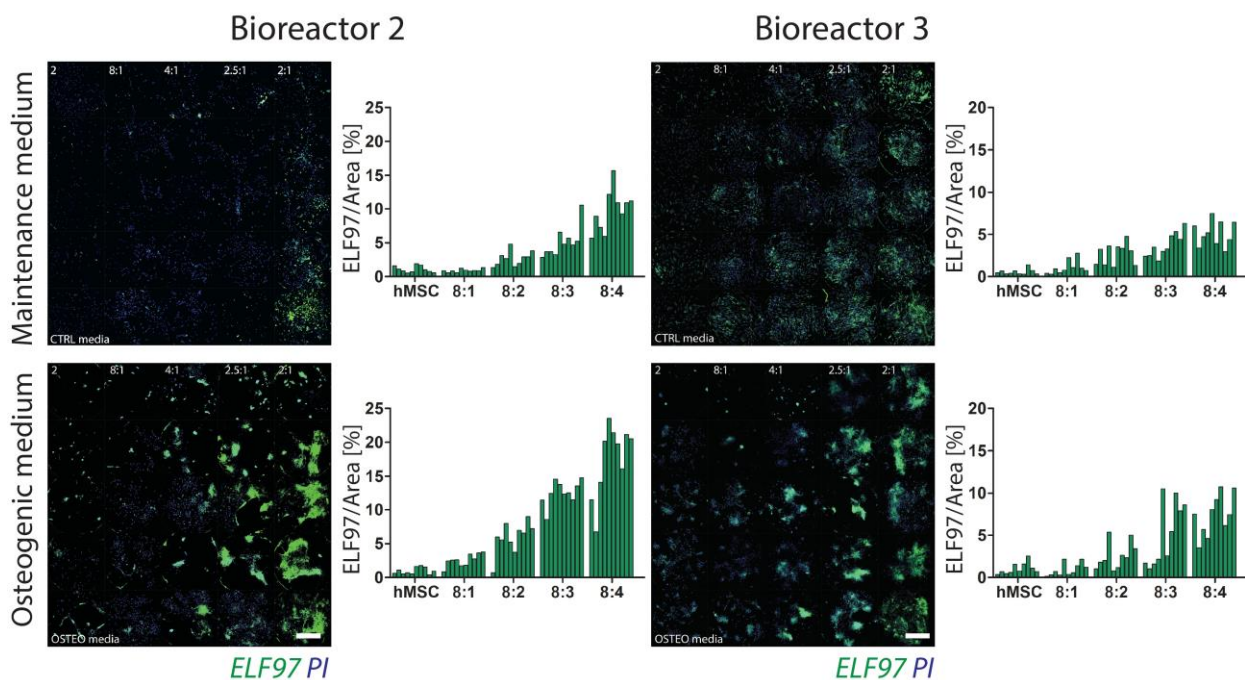


(b) SAOS2 low concentration gradient

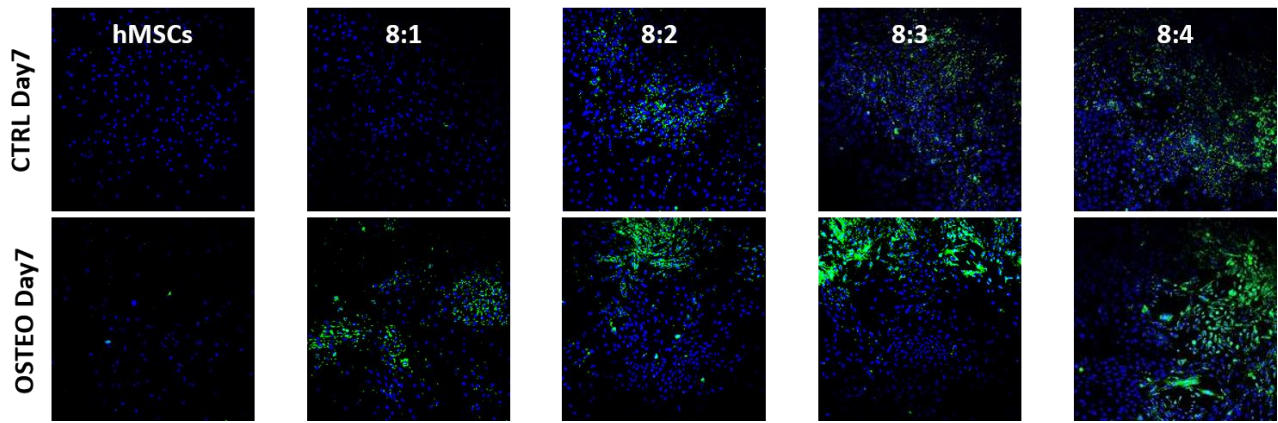


**Fig. SI2** Establishment of programmed hMSCs-SAOS2 osteogenic co-culture models within the microfluidic platform. Two different models were generated: a reverse gradient of hMSCs-SAOS2 (a) and a low concentration gradient of SAOS2 on a preseeded monolayer of hMSCs (b). The achievement of predicted mixing ratios is qualitatively visualized by DiD-staining of hMSC.

## Alkaline phosphatase activity



**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 (2 μl/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.