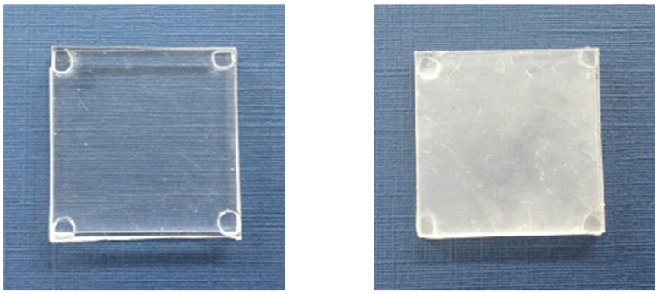
Supplementary Information for

Design and Assessment of a Microfluidic Network System for Oxygen Transport in an Engineered Tissue

Prevention of oxygen diffusion by the Parylene-C coating:

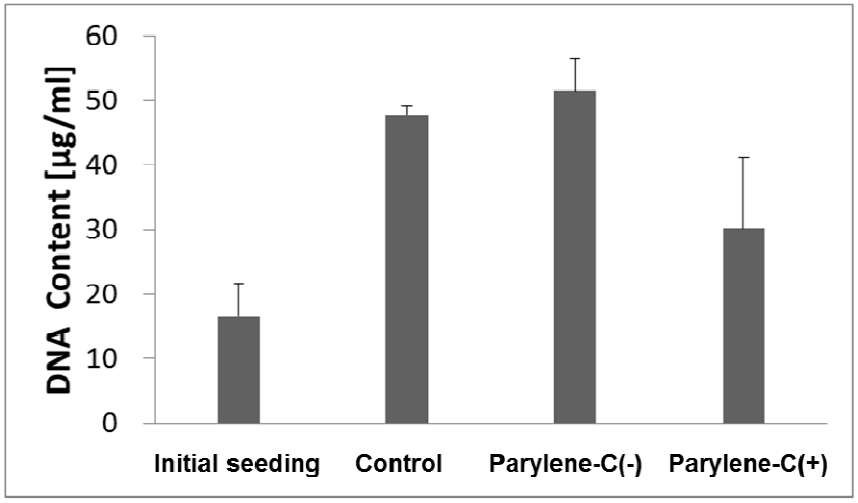
The PDMS chambers were coated with a 3-μm-thick Parylene-C film to block oxygen diffusion. The effect of the Parylene-C coating was confirmed by cell culture experiments. PDMS chambers for static culture were prepared with and without a Parylene-C coating (Supplementary Fig. 1). Cell-seeded scaffolds were inserted in each chamber and placed in well-plates with culture medium. Additionally, cell-seeded scaffolds were cultured without chambers as a control. After 5 days of culture, DNA contents were quantified, and the pimonidazole conjugation assay was performed to evaluate the effect of the Parylene-C coating. The DNA quantification results (Supplementary Fig. 2) showed that PDMS chambers without Parylene-C coating (Parylene-C(–)) did not hamper cell proliferation. The DNA content of the Parylene-C(–) group was not significantly different from that of the control group. However, PDMS chambers with the Parylene-C coating (Parylene-C(+)) had significantly reduced cell proliferation. The pimonidazole conjugation assay results (Supplementary Fig. 3) demonstrated that the low proliferation rate was due to the lack of oxygen. Most cells in the Parylene-C(–) group (Supplementary Fig. 3a) were stained with DAPI (blue) regardless of their position in the scaffold because they were supplied with

enough oxygen despite the PDMS plates. However, regional distribution of hypoxic and non-hypoxic areas was detected in the Parylene-C(+) group (Supplementary Fig. 3b). Cells residing in the interior part of the scaffold were stained with pimonidazole adduct (green), and the positive pimonidazole staining indicated that cells were at once alive under hypoxic conditions at oxygen concentrations <14 μM.

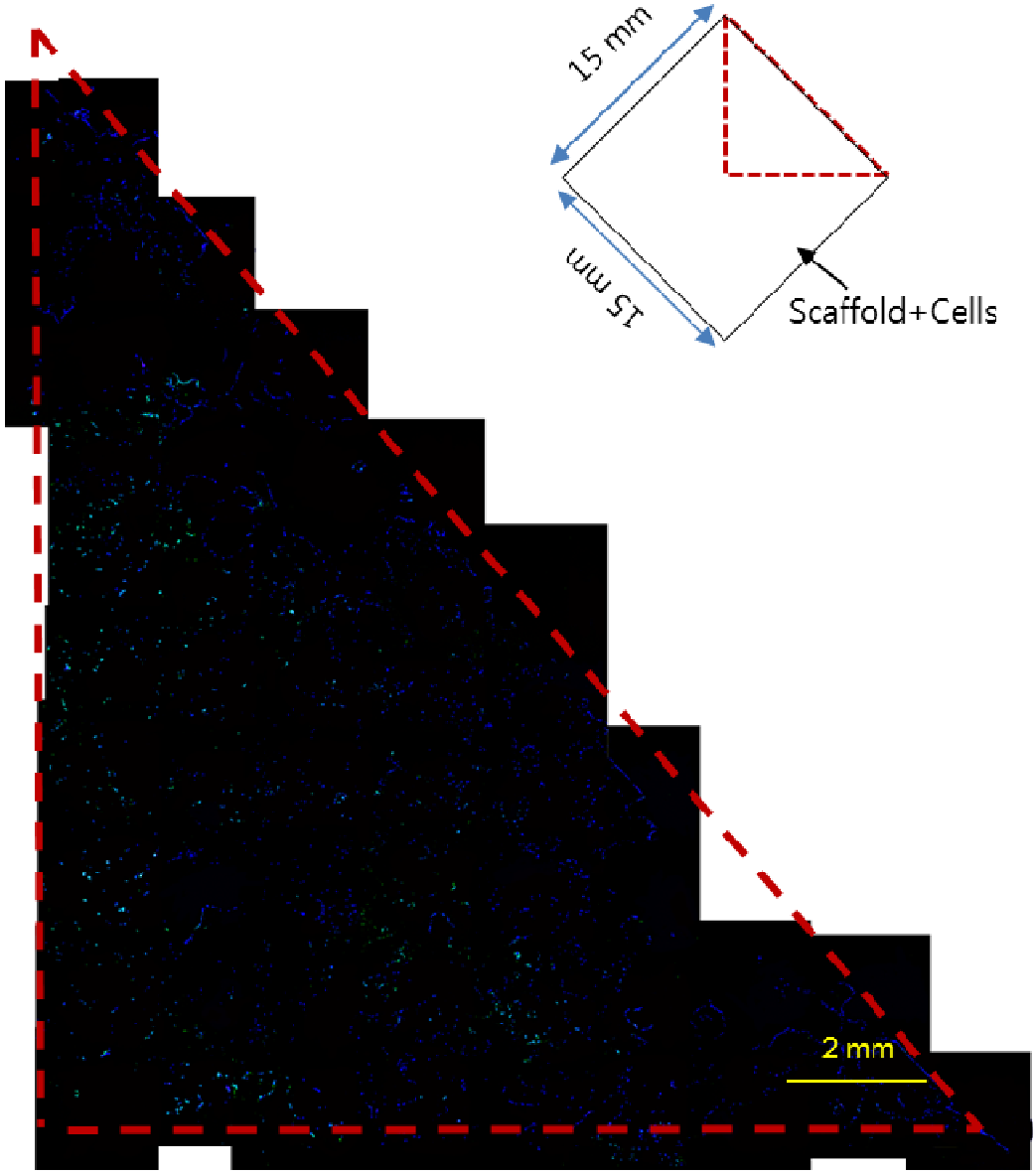


(a) (b)

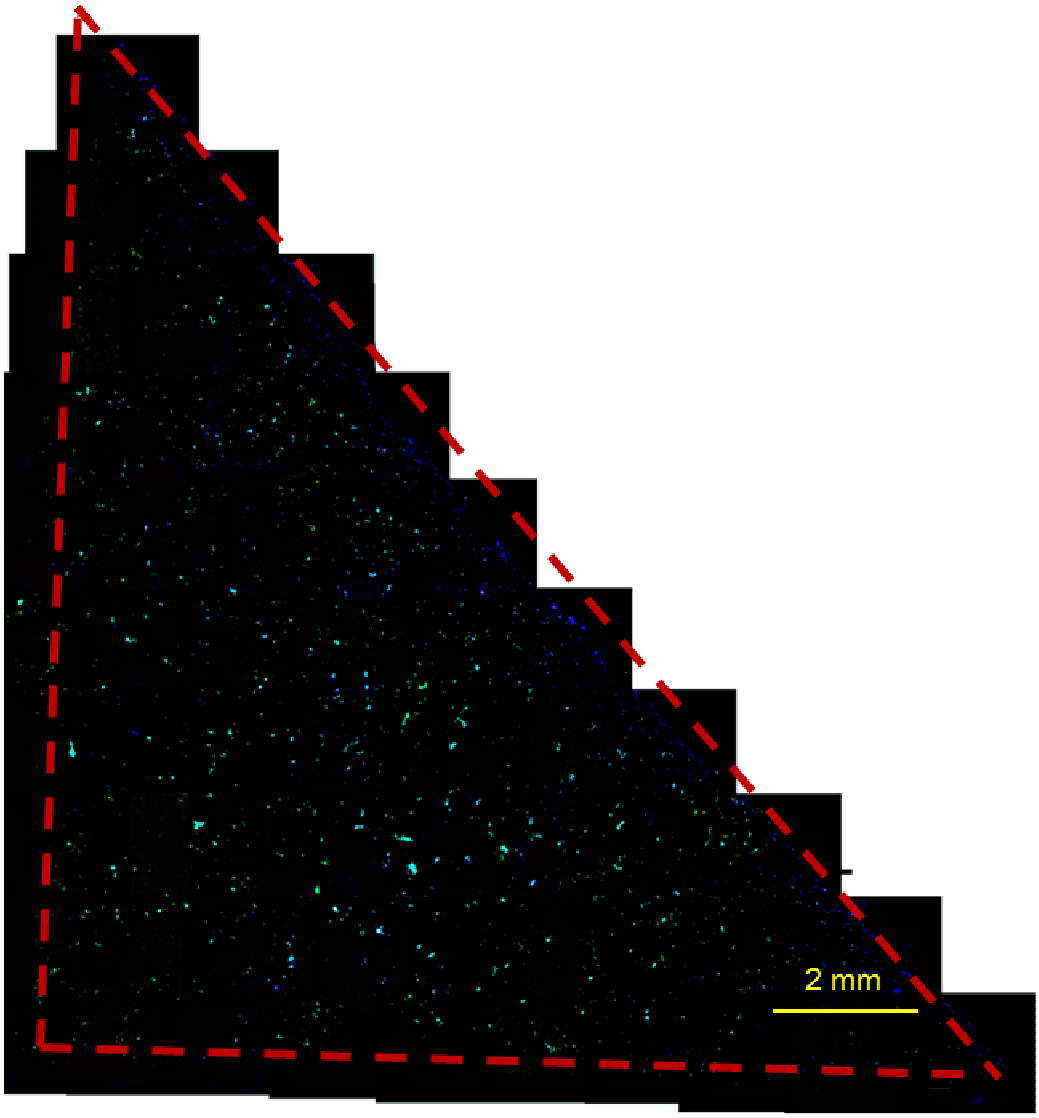
Supplementary Fig. 1. Polydimethylsiloxane (PDMS) chamber for static culture (a) without a Parylene-C coating and (b) with a Parylene-C coating.



Supplementary Fig. 2. Change in cell proliferation based on the presence of the Parylene-C coating.



(a)



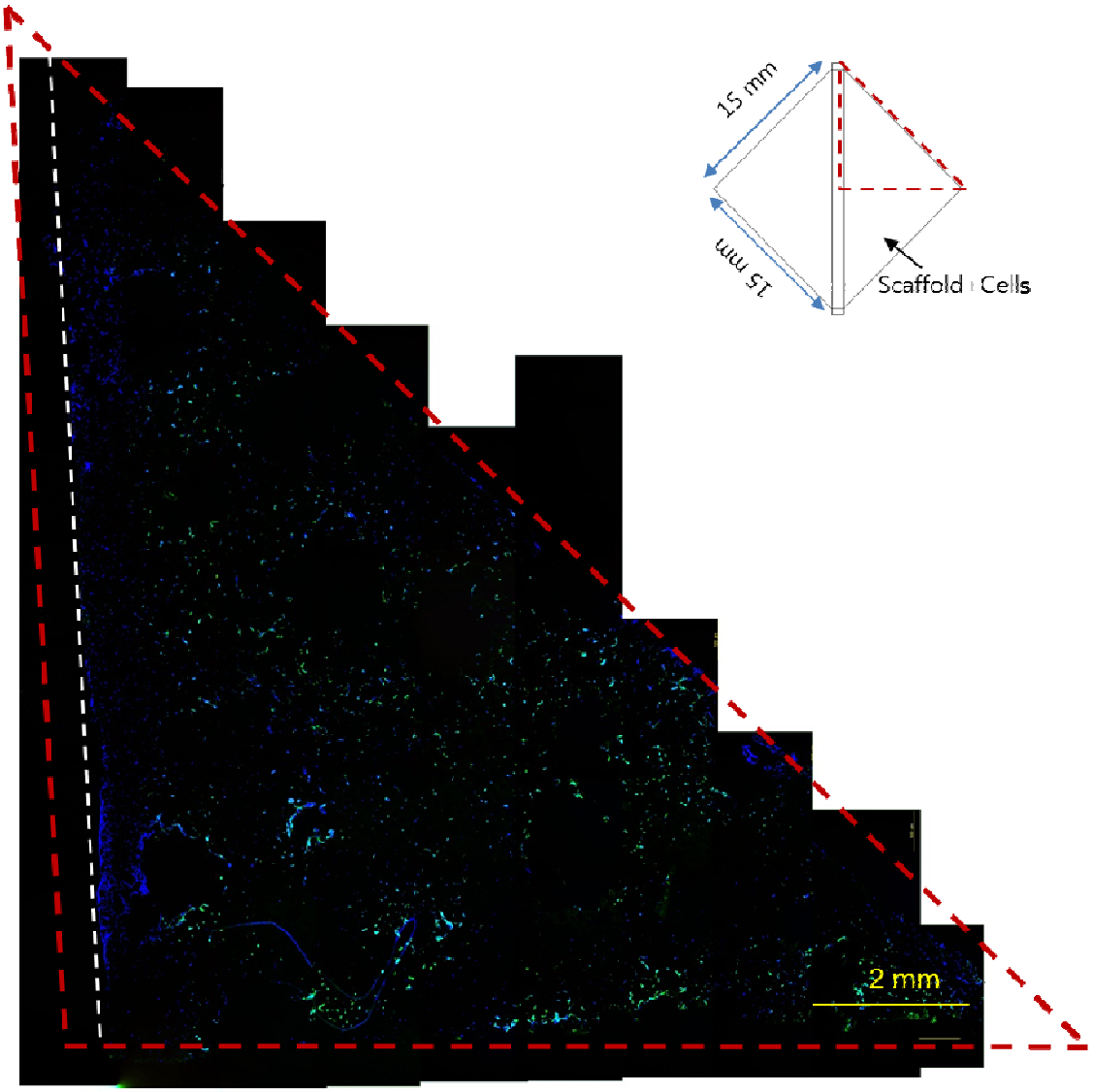
(b)

Supplementary Fig. 3. Distribution of hypoxic and non-hypoxic areas in (a) Parylene-C(–) and (b) parylene-C(+)-coated chambers.

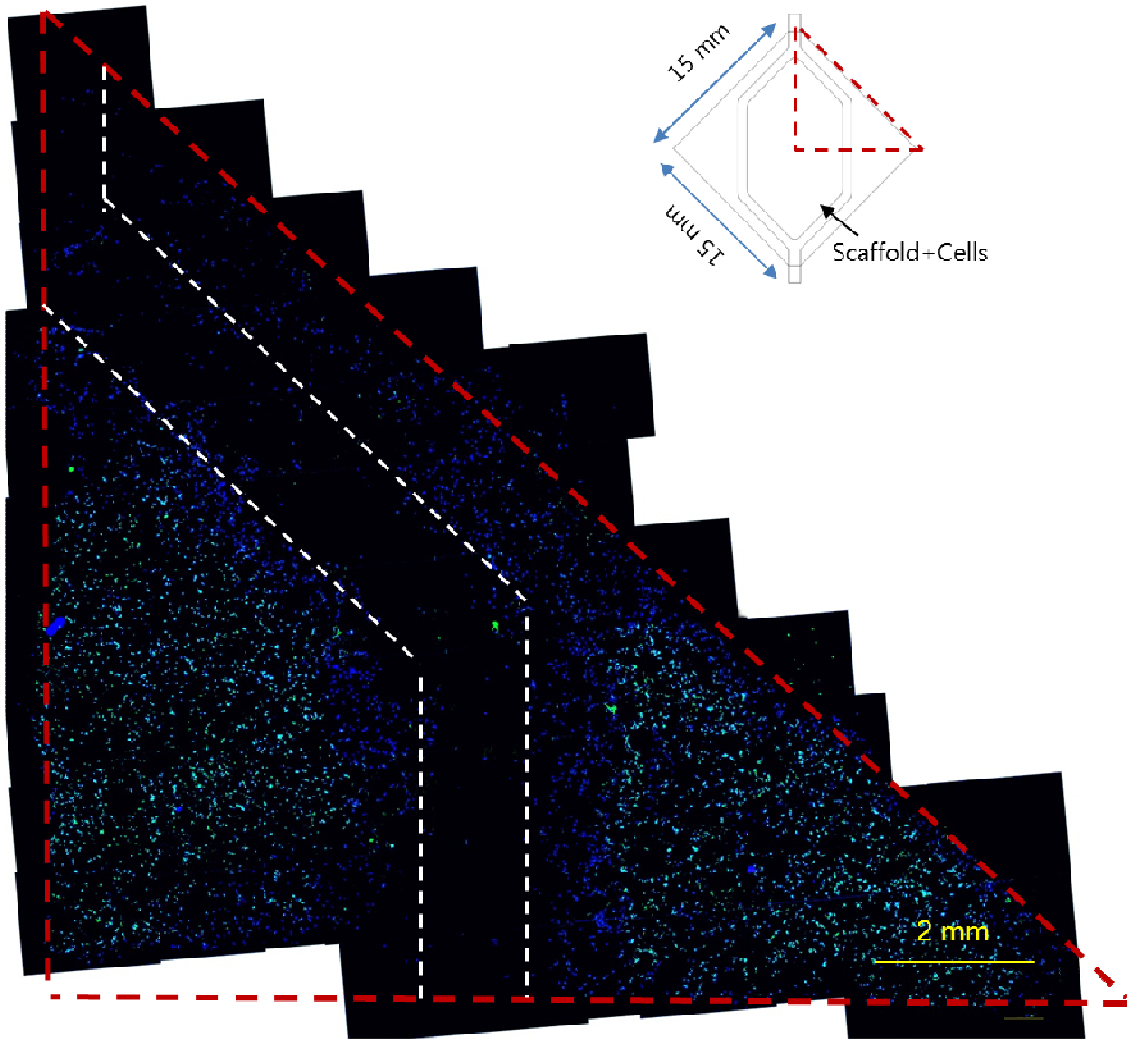
Overall distribution of the hypoxic area:

Regional distribution of the hypoxic and non-hypoxic areas was detected indirectly by the pimonidazole conjugation assay. Positive staining for pimonidazole adduct (green), which is presented with DAPI counterstaining (blue), indicated that cells were once alive under

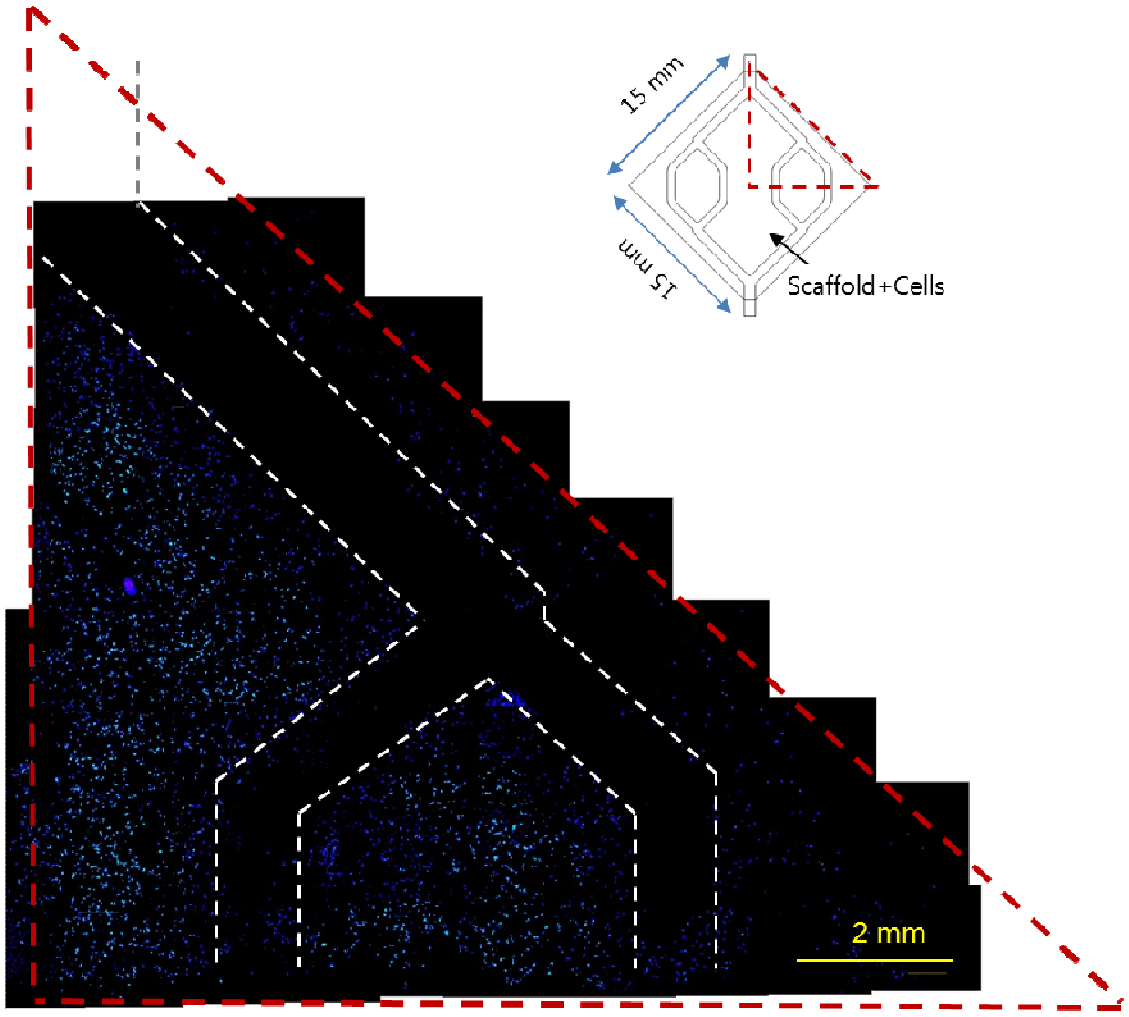
hypoxic conditions. To examine the overall distribution of the hypoxic area, multiple images (150–200) were aligned into one image for each model as follows (Supplementary Fig. 4a–d).



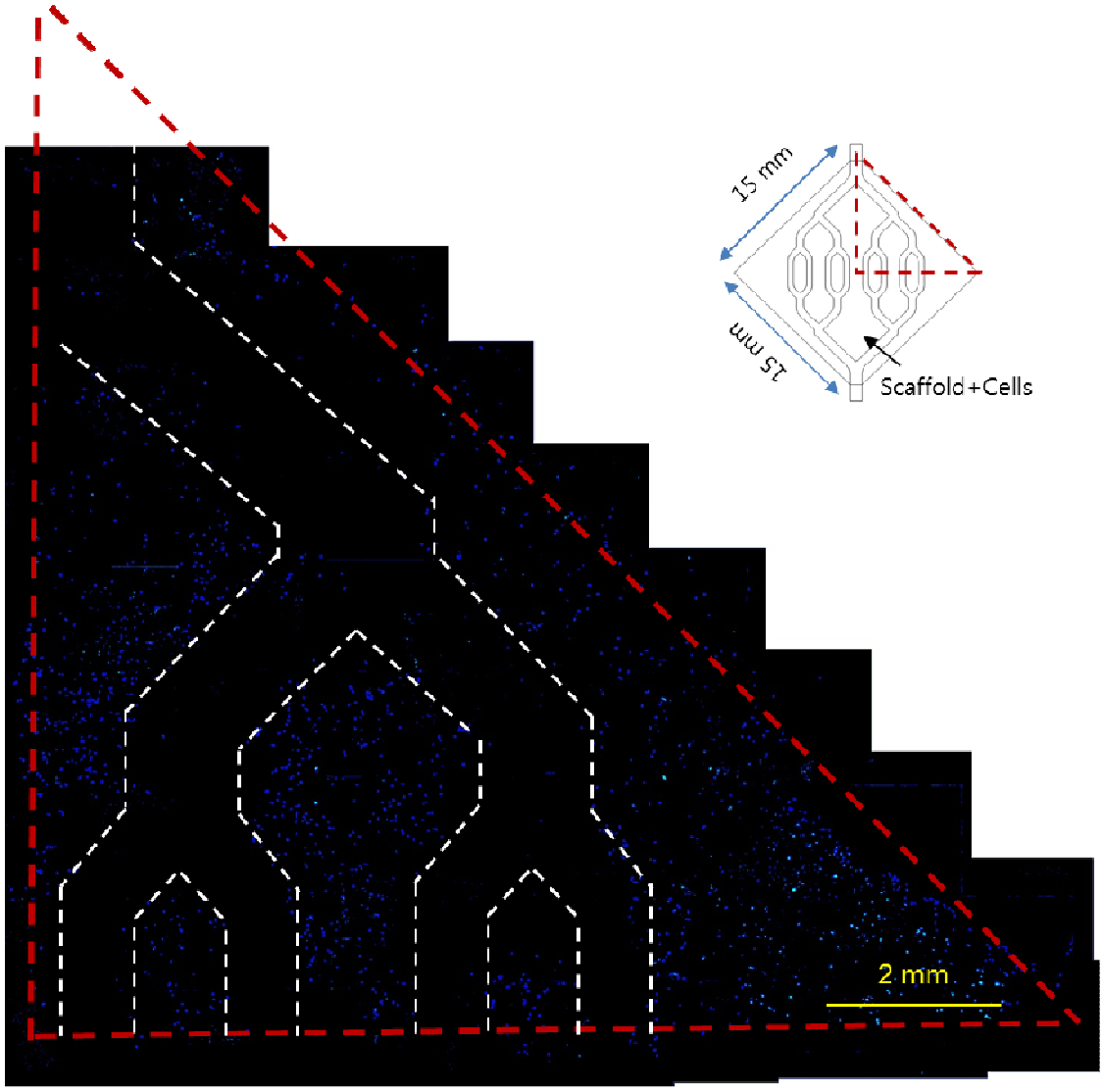
(a)



(b)



(c)



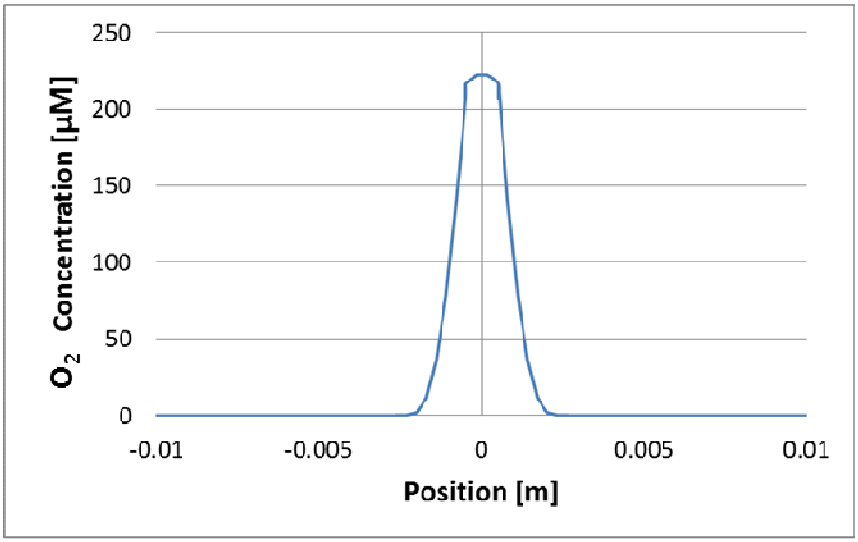
(d)

Supplementary Fig. 4. Overall distribution of hypoxic and non-hypoxic areas at (a) “Bifurcation: 0,” (b) “Bifurcation: 1,” (c) “Bifurcation: 2,” and (d) “Bifurcation: 3.”

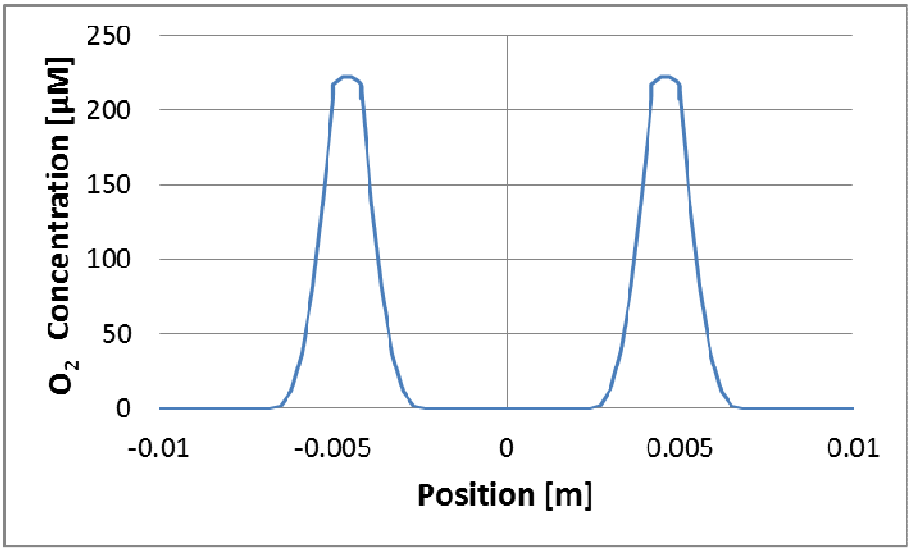
Simulated oxygen concentration profiles:

The simulated oxygen distributions are shown in Fig. 4b. The following figures represent the

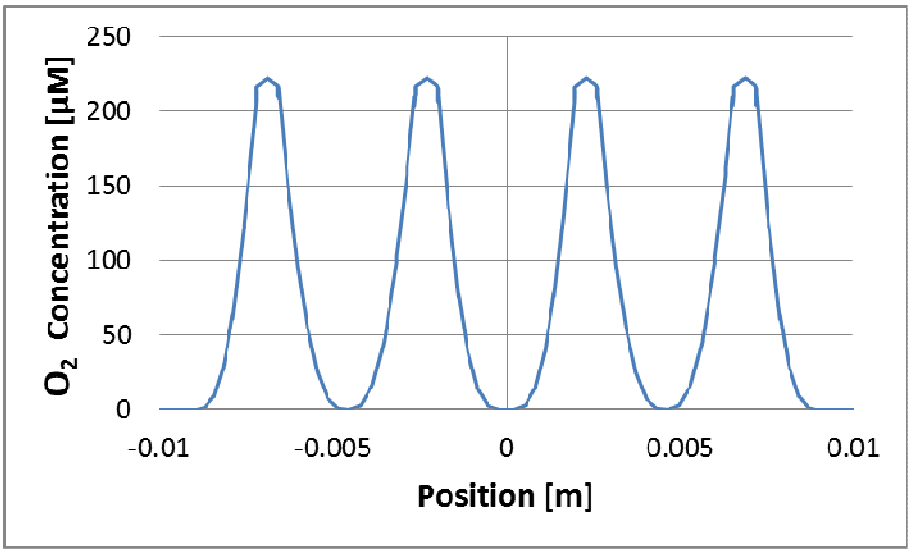
detailed oxygen concentration profiles along the central horizontal axis for each model.



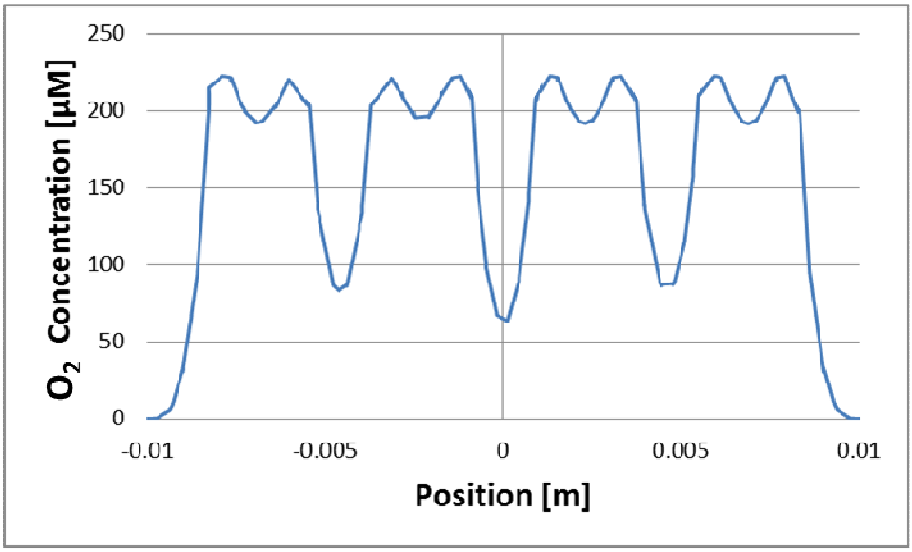
(a)



(b)



(c)



(d)

Supplementary Fig. 5. Simulated oxygen profile at (a) “Bifurcation: 0,” (b) “Bifurcation: 1,” (c) “Bifurcation: 2,” and (d) “Bifurcation: 3.”

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