Supplementary Information

Enhanced human T cell expansion with inverse opal hydrogels

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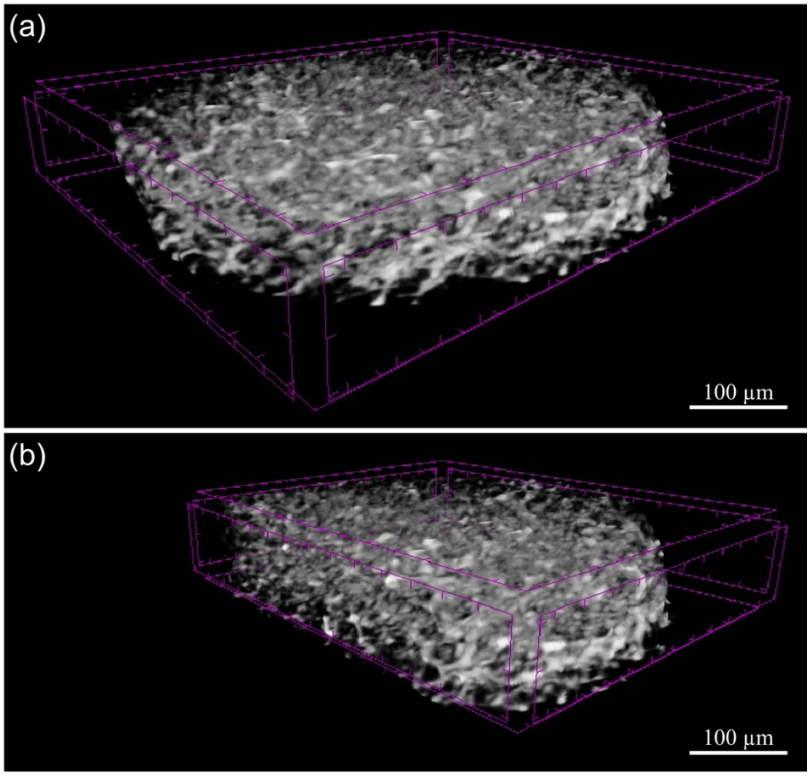
X-ray microtomography analysis



(c)

	Bulk 1		Bulk 2		IOPAL 1		IOPAL 2	
Volume of interest	9.7	mm^3	4.7	mm^3	12.0	mm^3	12.1	mm^3
Connectivity	51077		24546		252234		228569	
Connectivity density	5265.7	mm^{-3}	5222.6	mm^{-3}	21019.5	mm^{-3}	18890.0	mm^{-3}

Figure S1. X-ray microtomography analysis of 3D PEG-Hep hydrogels. a) X-ray microtomographs of a representative volume of interest and its b) cross-section of an IOPAL PEG-Hep hydrogel of 1 cm of diameter used to analyze its connectivity. c) Connectivity density obtained (connectivity/volume of interest) for 3D PEG-Hep bulk and IOPAL hydrogels ($N_{hydrogels} = 2$).

Strain sweeps and frequency sweeps of IOPAL PEG-Hep hydrogels

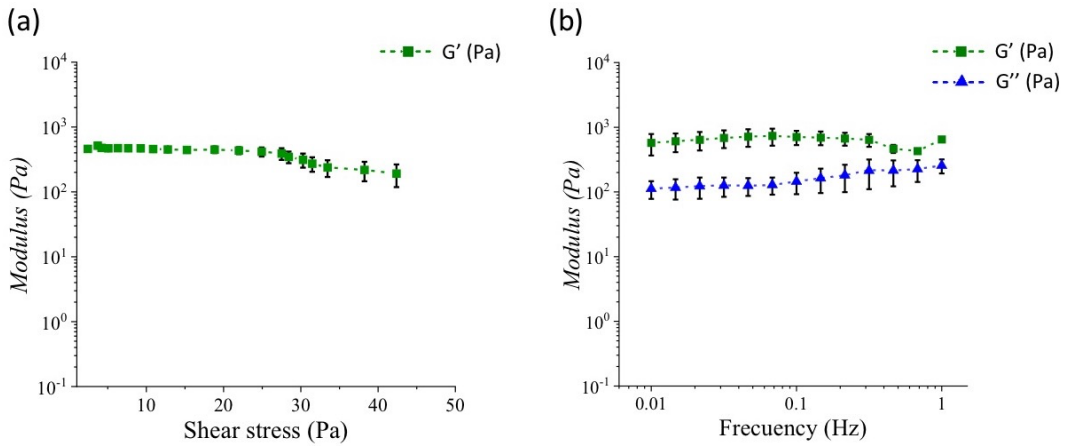


Figure S2. SAOS rheology. a) Strain and b) frequency sweeps for the IOPAL PEG-Hep hydrogels ($N_{Hydrogels}$ = 2). The results of the bulk PEG-Hep hydrogels were previously published by us.[1]

Formulas of the proliferation, expansion, and replication indexes

The software FlowJo was used to assess proliferation through a CFSE staining. The following three parameters were employed:[2]

Proliferation Index (PI):

$$PI = \frac{\sum_1^i i \times \frac{N_i}{2^i}}{\sum_1^i \frac{N_i}{2^i}}$$

Expansion Index (EI):

$$EI = \frac{\sum_0^i N_i}{\sum_0^i \frac{N_i}{2^i}}$$

Replication Index (RI):

$$RI = \frac{\sum_1^i N_i}{\sum_1^i \frac{N_i}{2^i}}$$

Raw data proliferation analysis of primary human CD4+ T cells in bulk and IOPAL PEG-Hep hydrogels

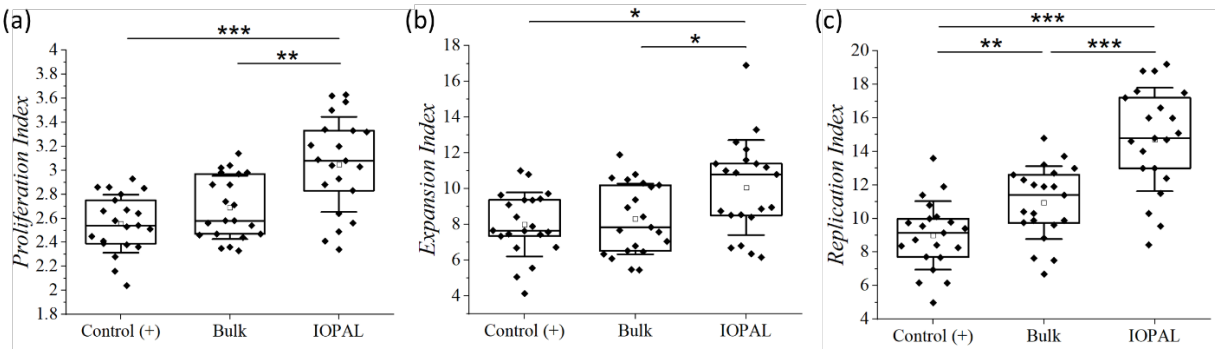


Figure S3. Proliferation analysis of primary human CD4+ T cells in bulk and IOPAL PEG-Hep hydrogels. Raw data of a) proliferation, b) expansion, and c) replication analysis of CD4+ T cells 6 days after seeding in bulk and IOPAL PEG-Hep hydrogels ($N_{donors} = 7$). Dynabeads were used to activate the cells. Statistical significance was determined by the Mann-Whitney U test (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$).

Differentiation dot plots for primary human CD4+ T cells cultured in bulk and IOPAL PEG-Hep hydrogels

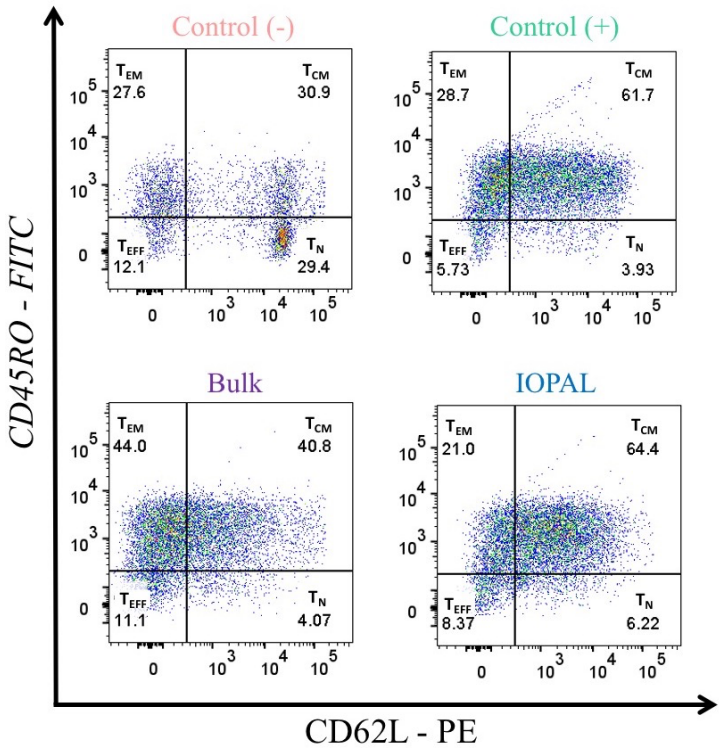


Figure S4. Representative differentiation dot plots for primary human CD4+ T cells cultured in bulk and IOPAL PEG-Hep hydrogels. Percentage of naïve (T_N), central memory (T_{CM}), effector (T_{EFF}), and effector memory (T_{EM}) CD4+ T cells seeded on bulk and IOPAL PEG-Hep hydrogels on day 5, represented in CD45RO-FITC vs CD62L-PE differentiation dot plots. The negative control consists of cells seeded in suspension without Dynabeads, whereas in the positive control, cells are seeded with Dynabeads. When cells are seeded in the hydrogels, they are always stimulated with Dynabeads.

Differentiation of primary human CD4+ T cells cultured in bulk and IOPAL PEG-Hep hydrogels

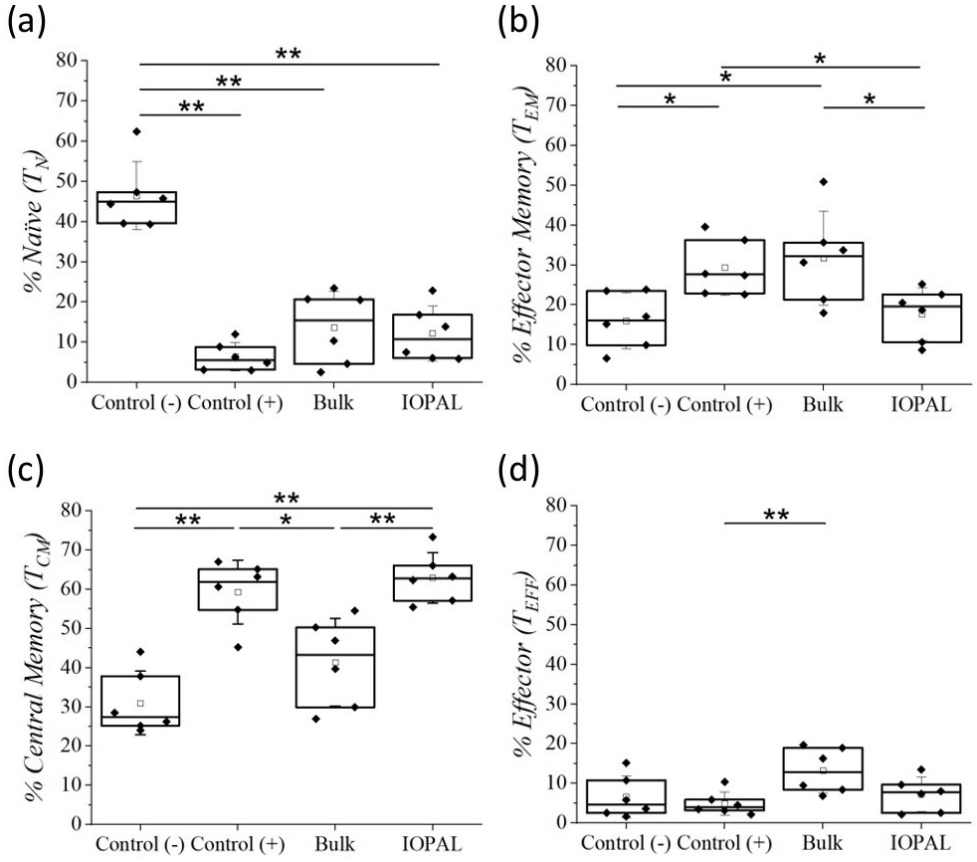


Figure S5. Differentiation of primary human CD4+ T cells in bulk and IOPAL PEG-Hep hydrogels. a) Percentage T_N, b) T_{EM}, c) T_{CM}, d) T_{EFF} CD4+ T cells on day 5 (N_{donors} = 6). The negative control consists of cells seeded in suspension without Dynabeads, whereas in the positive control, cells are seeded with Dynabeads. When cells are seeded in the hydrogels, they are always stimulated with Dynabeads. Statistical significance was determined by the Mann-Whitney U test (*$p < 0.05$, **$p < 0.01$).

References

1 E. Pérez del Río, F. Santos, X. R. Rodriguez, M. Martínez-Miguel, R. Roca-Pinilla, A. Arís, E. Garcia-Fruitós, J. Veciana, J. P. Spatz, I. Ratera and J. Guasch, *Biomaterials*, 2020, **259**, 120313.

2 M. Roederer, *Cytom. A*, 2011, **79A**, 95-101.