Supporting Information

3D Printing as a Strategy to Scale-Up Biohybrid Hydrogels for T Cell Manufacture

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1. Differentiation analysis of primary human CD4+ T cells

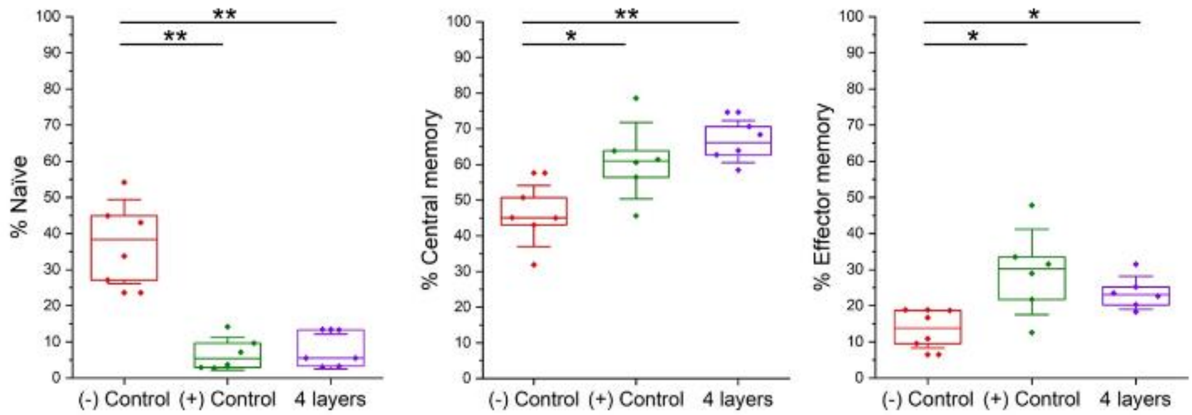

Figure S1. Differentiation analysis of primary human CD4+ T cells seeded in printed PEG-Hep hydrogels of 4 layers of height with their respective controls (N_{donors} = 6). Statistical significance was determined by the Mann-Whitney U test (*p<0.05, **p<0.01).

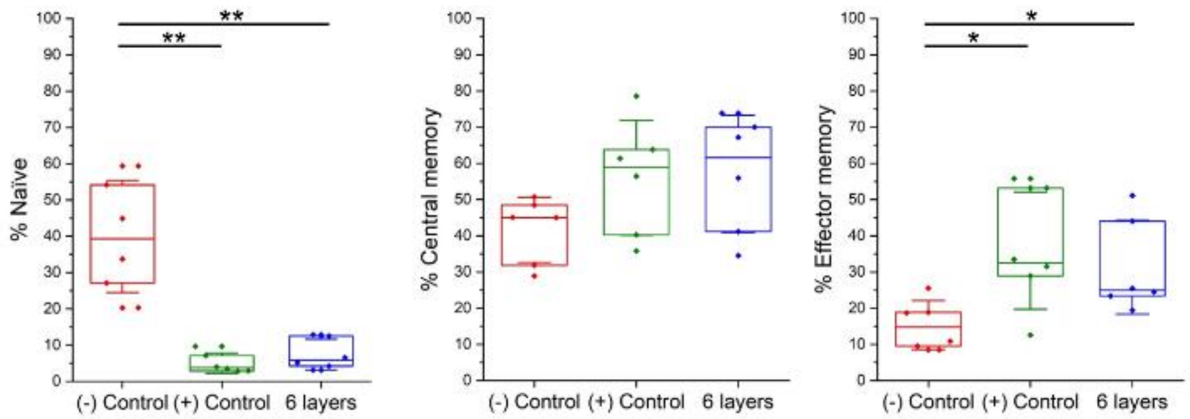

Figure S2. Differentiation analysis of primary human CD4+ T cells seeded in printed PEG-Hep hydrogels of 6 layers of height with their respective controls (N_{donors} = 6). Statistical significance was determined by the Mann-Whitney U test (*p<0.05, **p<0.01).

2. Mechanical properties of 3D printed hydrogels

The mechanical properties of the resulting scaffold were measured by rheology and compared with the non-printed material.[1] Strain sweeps were performed at a constant frequency of 1.0 Hz and the pressure was swept from 1 Pa to 50 Pa on the printed hydrogel and compared with the non-printed hydrogel (Figure S3). The linear behavior of the dynamic modulus (G′) of the printed hydrogel is within the strength range of 1–10 Pa, which is a lower value than the linear behavior from 10–100 Pa of the non-printed hydrogel.

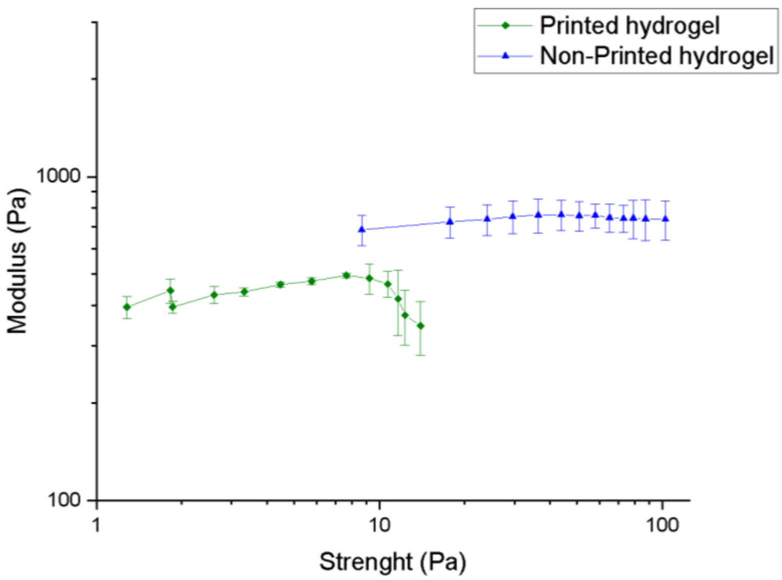

Figure S3. Strain sweeps at a constant frequency of 1.0 Hz to compare the mechanical properties of the hydrogel printed and non-printed.

The equilibrium shear modulus (G_e) achieved for the printed hydrogel was of 447 ± 34 Pa in comparison with the 1.1 ± 0.1 KPa of the non-printed hydrogel.

3. 3D printing for small size hydrogels

In these experiments, we compared the proliferation results obtained with 6-layer 3D printed hydrogels and with non-printed hydrogel (N.P. 3%wt) of the approximately the same mass (~50 µg). The results can be seen in Figure S4.

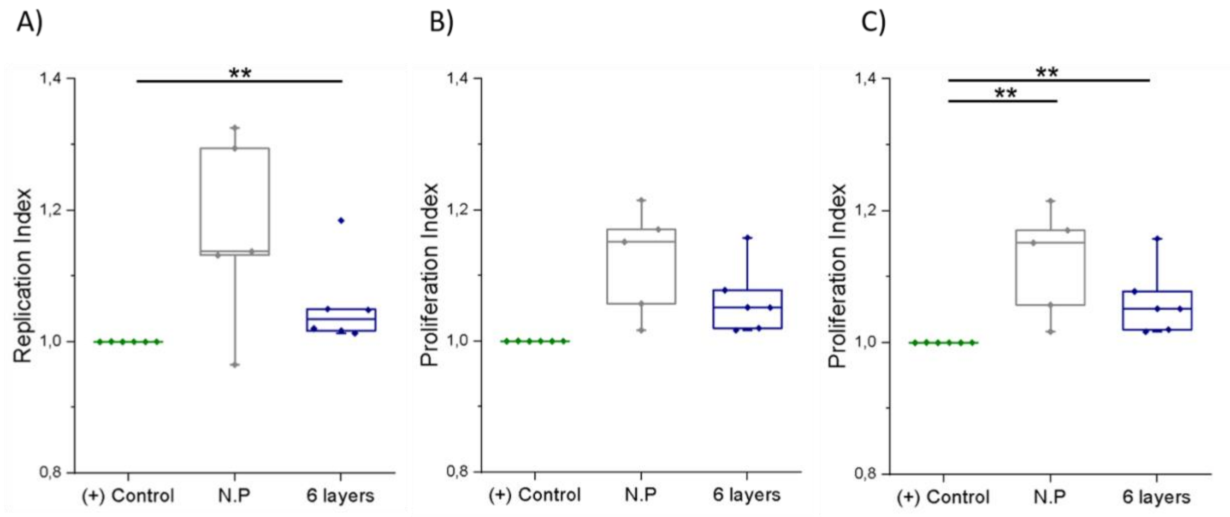

Figure S4. Normalized proliferation analysis of primary human CD4+ T cells seeded in suspension (positive control), non-printed (N.P., bulk) PEG-heparin hydrogels and 6-layer 3D printed hydrogels 6 days after seeding A) Replication, B) expansion, and C) proliferation indexes (N_{donors} = 6). Statistical significance was determined by the Mann-Whitney U test (*p<0.05, **p<0.01).

The non-printed PEG-heparin hydrogels improved the proliferation indexes ca. 15% (i.e. mean values of 1.15) compared to the positive control, although these changes were only statistically relevant in the case of the proliferation index. These results evidenced that, at this scale of production, the used non-printed hydrogels allow a better transfer of cells, waste, nutrients, and gases.

4. Reference

(1) Pérez del Río, E.; Santos, F.; Rodriguez, X. R.; Martínez-Miguel, M.; Roca-Pinilla, R.; Arís, A.; Garcia-Fruitós, E.; Veciana, J.; Spatz, J. P.; Ratera, I.; Guasch, J. CCL21-loaded 3D hydrogels for T cell expansion and differentiation. *Biomaterials* **2020**, *259*, 120313.