ADVANCED HEALTHCARE MATERIALS

Supporting Information

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3D printed dual-porosity scaffolds: the combined effect of stiffness and porosity in the modulation of macrophage polarization

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Supporting Information

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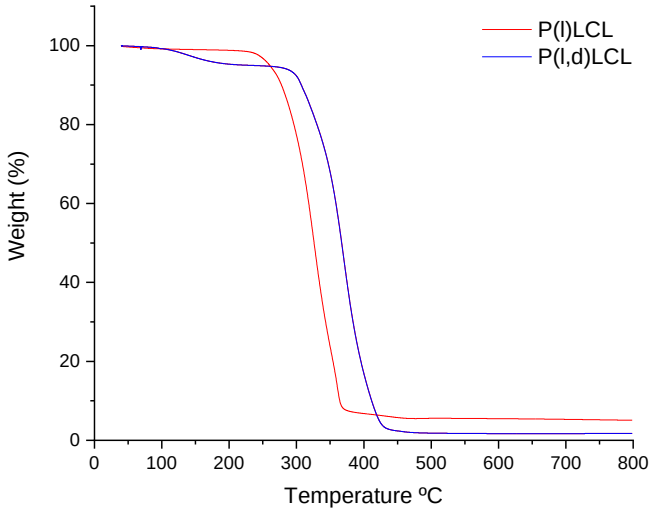
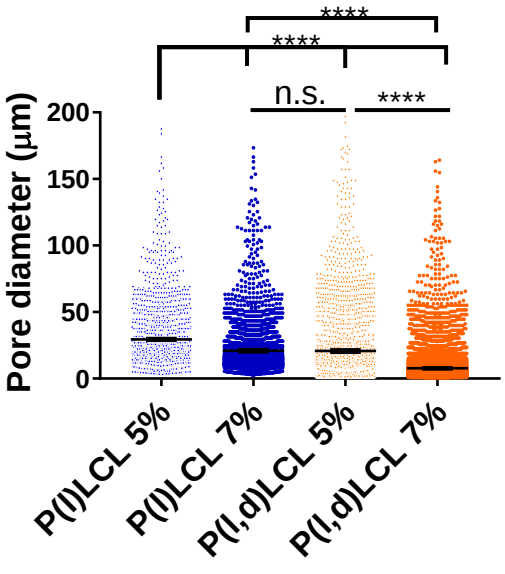
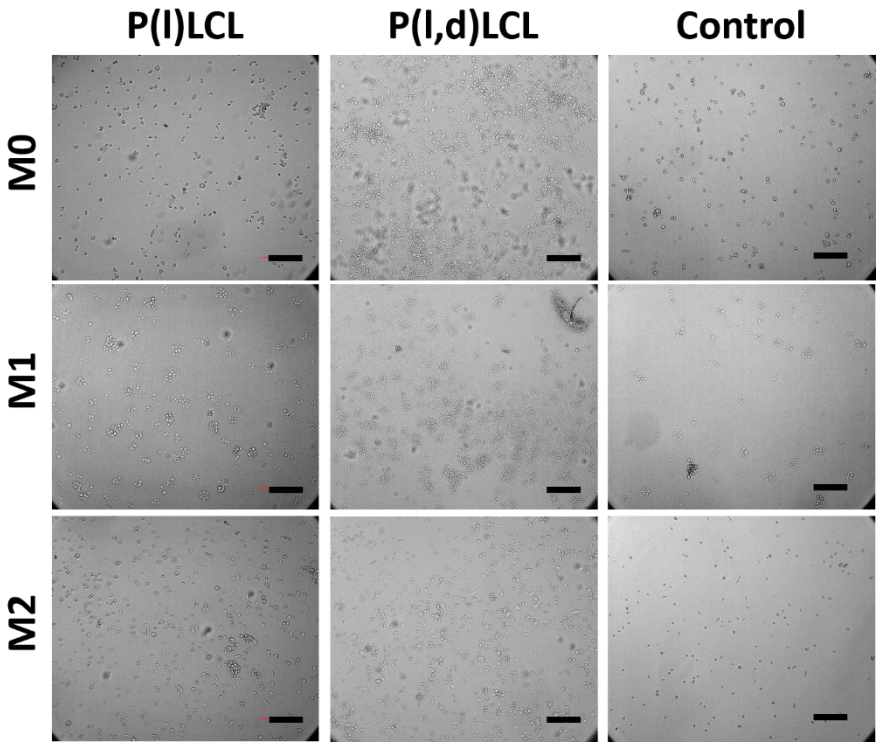


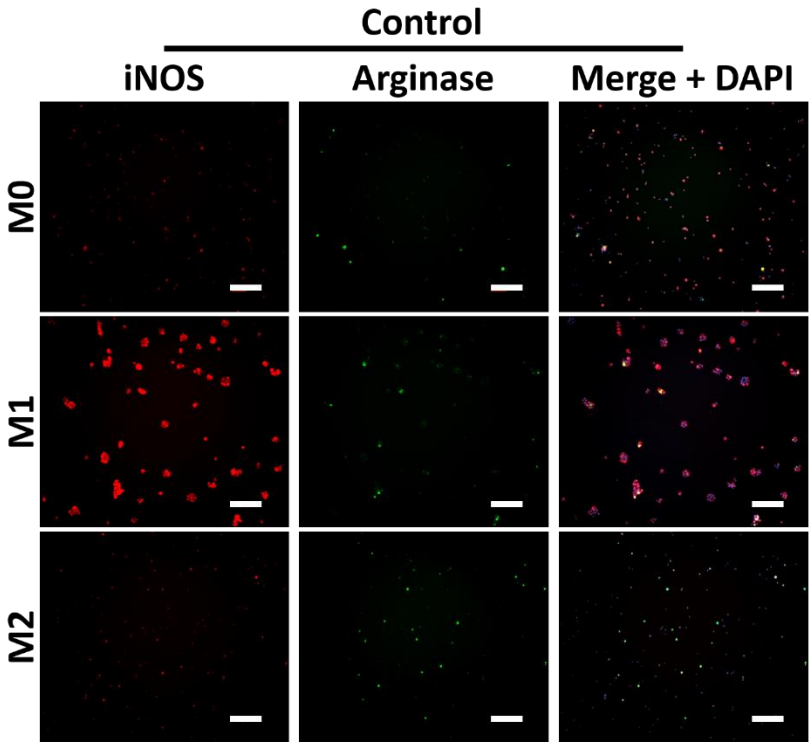
Supporting Information Figure 1. Thermogravimetric analysis of P(l)LCL and P(l,d)LCL under nitrogen atmosphere, sowing a degradation onset of approximately 300°C and 350° C, respectively.



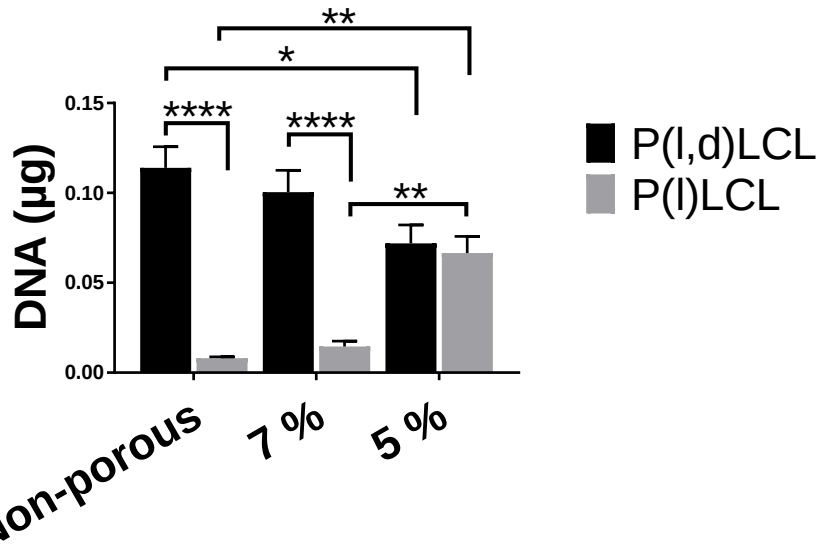
Supporting Information Figure 2. Pore diameter on dual porosity scaffolds. Error bars represent mean with 95 % CI. Statistical significance was calculated from one-way ANOVA with Tukey's multiple comparison test. (****) $p<0.0001$, (***) $p<0.001$, (**) $p<0.01$ and (*) $p<0.1$.



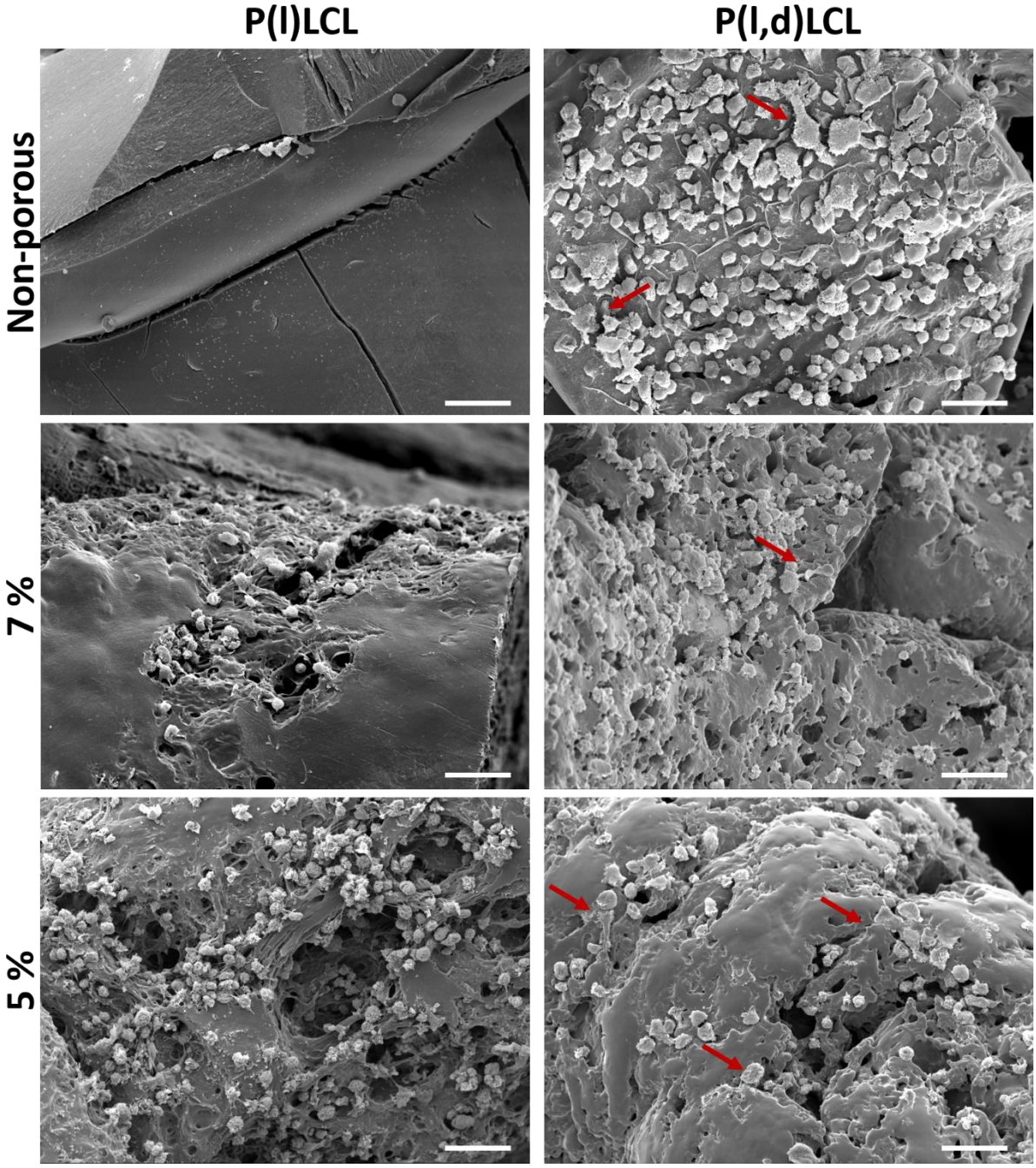
Supporting Information Figure 3. Bright field images of macrophages attached to 2D spin coated substrates of P(l)LCL, P(l,d)LCL and TCP control after 48 h of culture. M0 represents the native state of cells when attached to the substrate. M1 (LPS + IFN-γ) and M2 (IL-13 + IL-4) are macrophages after polarization with towards the corresponding phenotypes. Scale bar in all images is 200 µm.



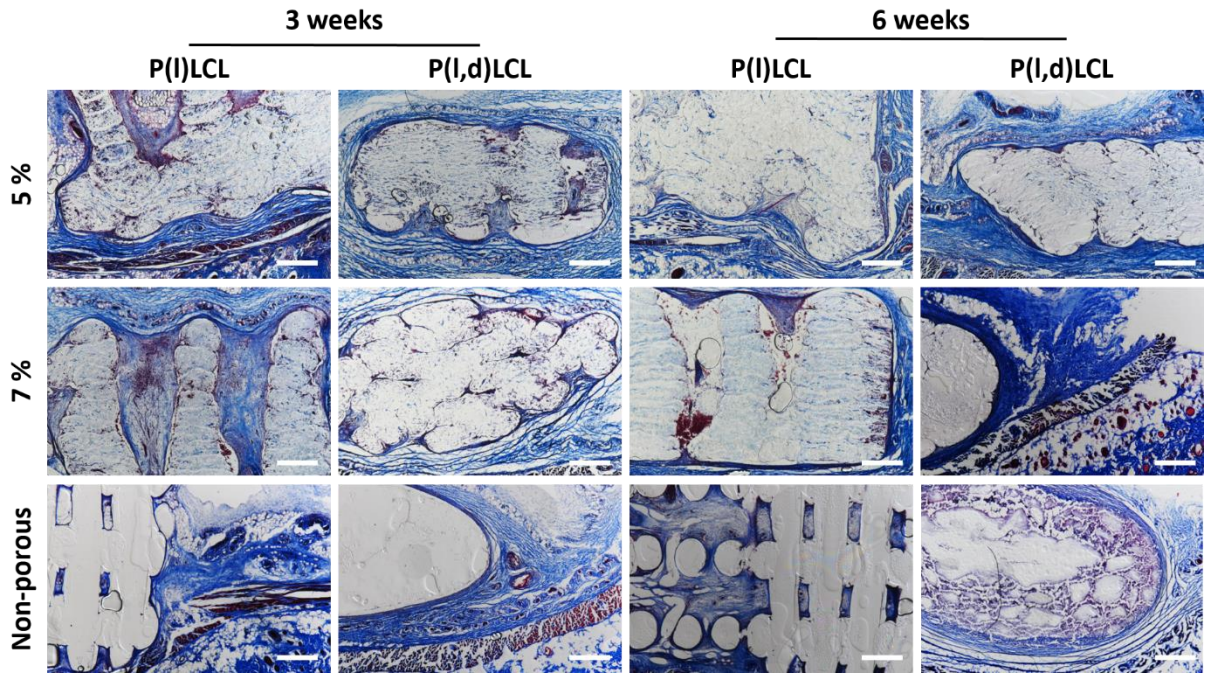
Supporting Information Figure 4. Fluorescence microscopy images of macrophages attached to TCP control after 48 h of culture. M0 represents the native state of cells when attached to the substrate. M1 (LPS + IFN-γ) and M2 (IL-13 + IL-4) are macrophages after polarization with towards the corresponding phenotypes. Cells were stained for iNOS (red), arginase (green) and DNA (DAPI, blue) Scale bar in all images is 200 µm.



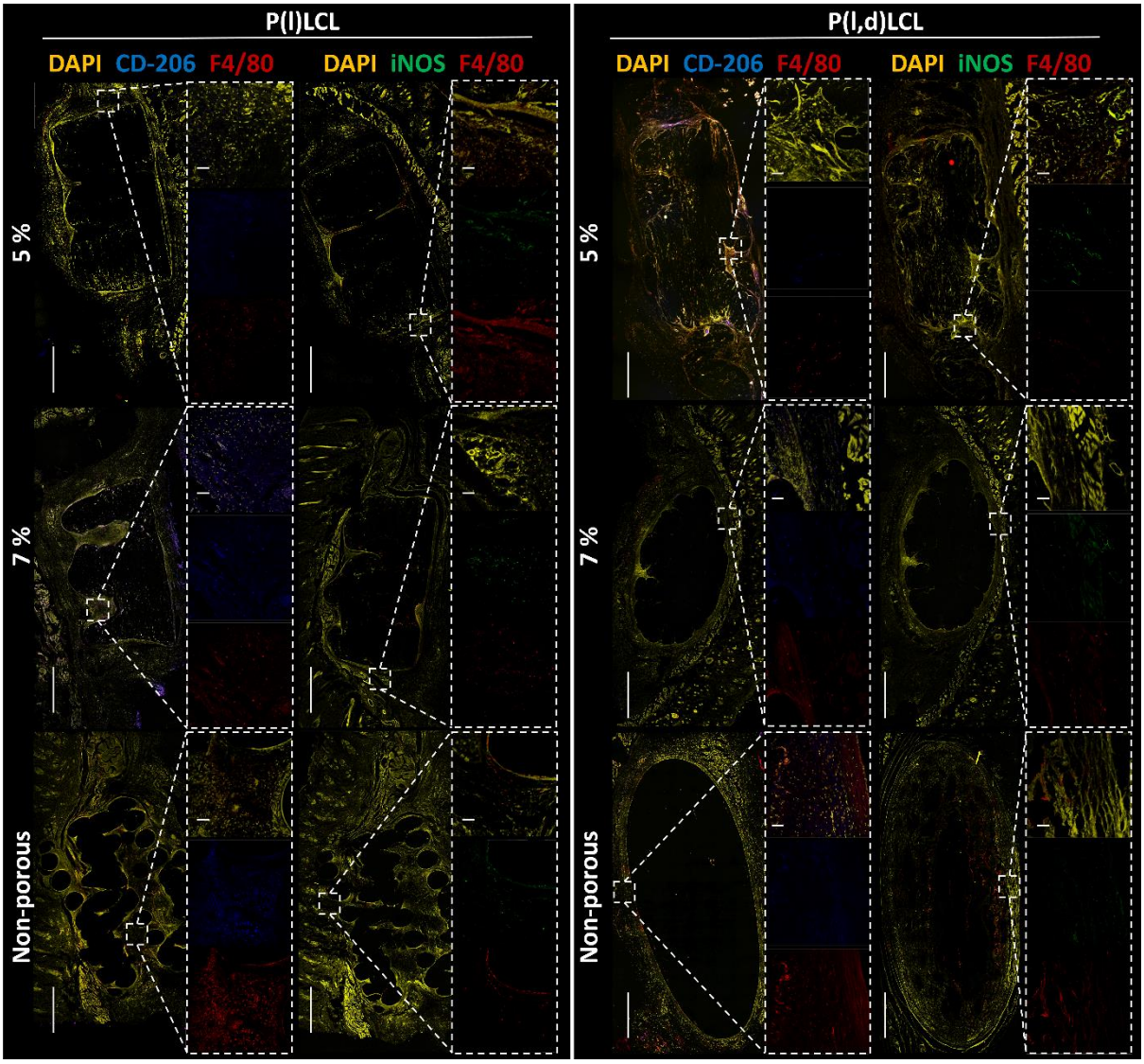
Supporting Information Figure 5. Cell attachment to 3D scaffolds. DNA quantification after 48h of culture in non-porous and dual-porosity scaffolds produced from 7% and 5% gels of P(l,d)LCL and P(l)LCL. Statistical significance was calculated from two-way ANOVA with Tukey's multiple comparison test. (****) $p<0.0001$, (***) $p<0.001$, (**) $p<0.01$ and (*) $p<0.1$.



Supporting Information Figure 6. Scanning electron microscopy images of macrophages cultured in FDM (non-porous) scaffolds and, 7% and 5%dual-porosity scaffolds fabricated from P(l)LCL or P(l,d)LCL copolymers. Red arrows indicate flattened cells. Scale bar is 50 µm.



Supporting Information Figure 7. Optical microscopy images of non-porous scaffolds and, 7% and 5% dual-porosity scaffolds fabricated from P(l)LCL or P(l,d)LCL copolymers after in-vivo implantation for 3 and 6 weeks. Scale bar is 500 µm.



Supporting Information Figure 8. Macrophage polarization state after 6 weeks of in-vivo implantation. Immunofluorescence staining of macrophages in the proximity to non-porous and porous P(l)LCL or P(l,d)LCL scaffolds prepared from 7% and 5% polymer gels. Macrophages were stained for F4/80 (red) and mannose receptor (CD-206, blue, M2) or iNOS (green, M1). The entire section was also stained for DAPI (nucleus, yellow) to identify other cells. Scale bar in all images is 1 mm. Dash line boxes indicate areas were a zoom-in was taken. Scale bar in zoom-in images is 30 µm.

Table 1. Porosity of scaffolds from µCT scans

Sample	Porosity (%)	Closed Porosity (%)	Total surface area (mm^2)	Total pore volume (mm^3)
P(l)LCL 5%	61,53	0,01	3103	30,8
P(l)LCL 7%	69,16	0,07	2020	34,6
P(l,d)LCL 5%	53,33	0,01	3543	26,6
P(l,d)LCL 7%	60,97	0,03	2548	30,5