Supporting information

Sustained release of dexamethasone from 3D printed scaffolds modulates macrophage activation and enhances osteogenic differentiation

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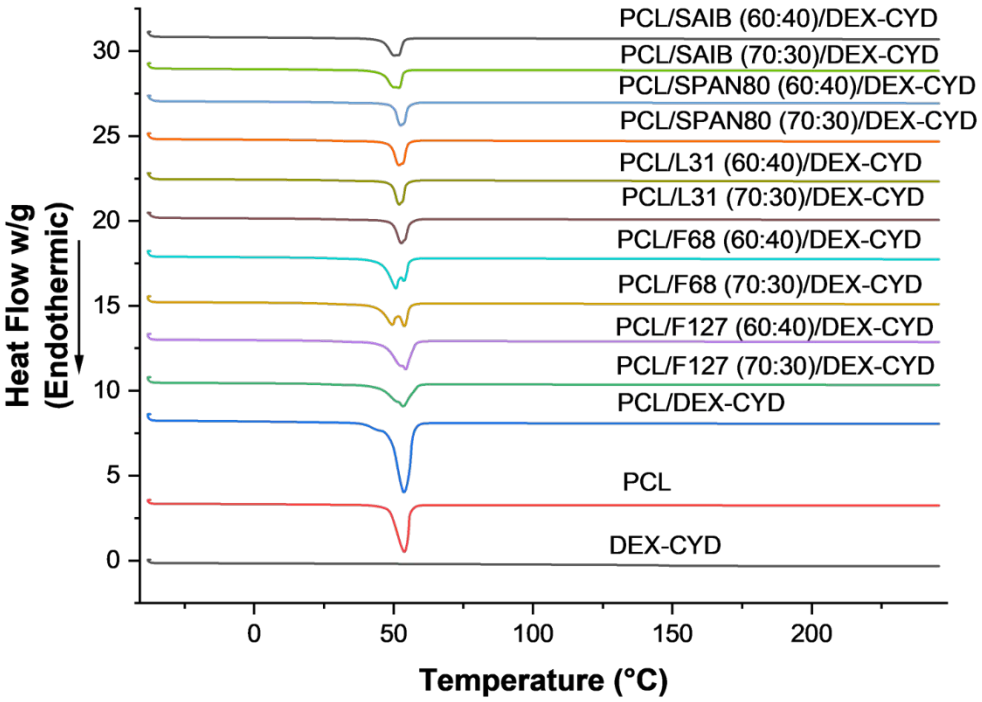

Figure S1: Differential scanning calorimetry thermograms of different ink formulations.

Table S1: Parameters determined by DSC for different ink formulations.

Sample	T_m (°C)	ΔH_m	X_c (%)
PCL	57.53	65.450	46.92
PCL/DEX-CYD	56.08	84.36	60.47
PCL/F127 (70:30)/DEX-CYD	53.57	59.21	42.44
PCL/F127 (60:40)/DEX-CYD	54.17	67.56	48.43
PCL/F68 (70:30)/DEX-CYD	54.06	58.89	42.22
PCL/F68 (60:40)/DEX-CYD	52.38	69.29	49.67
PCL/L31 (70:30)/DEX-CYD	52.67	39.78	28.52
PCL/L31 (60:40)/DEX-CYD	51.87	36.35	26.06
PCL/Span80 (70:30)/DEX-CYD	51.12	39.96	28.85
PCL/Span80 (60:40)/DEX-CYD	50.04	37.46	26.85
PCL/SAIB (70:30)/DEX-CYD	51.98	39.17	28.08
PCL/SAIB (60:40)/DEX-CYD	49.71	36.19	25.94

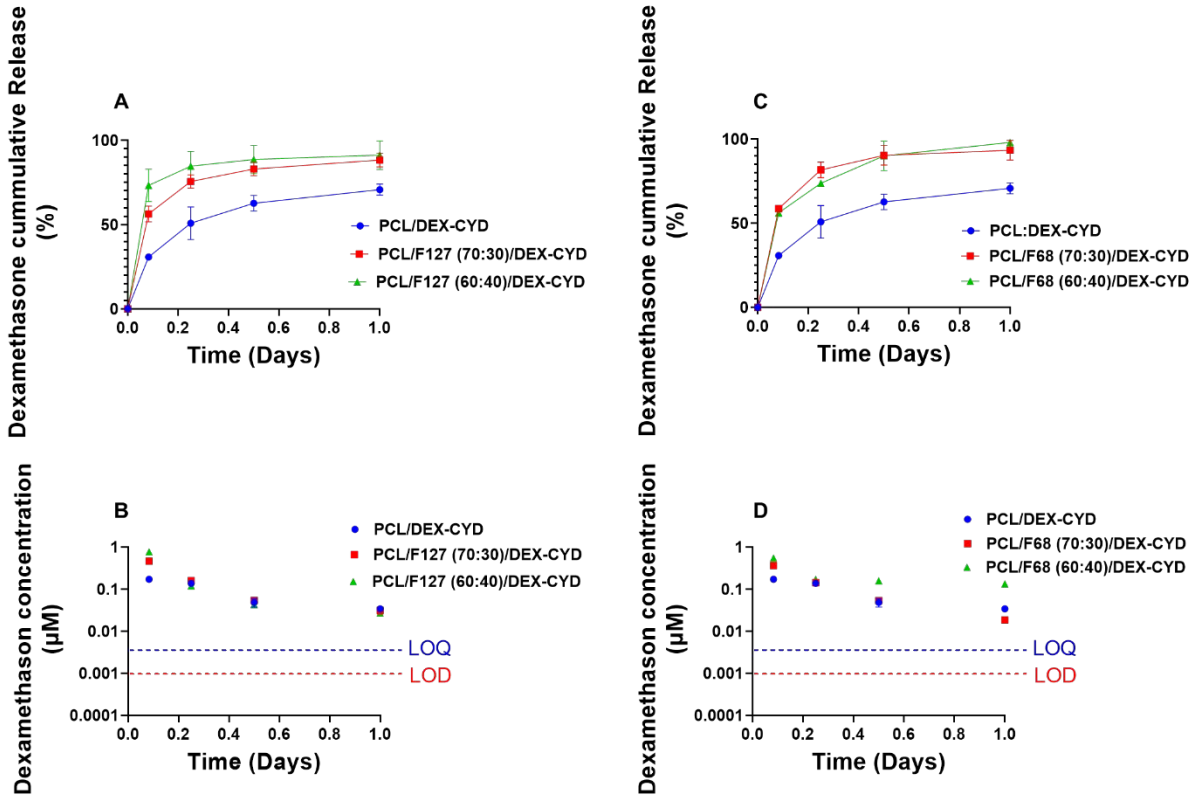

Figure S2: Cumulative and individual release at each collection time point from 3D printed scaffolds in 10mL PBS. (**A**) Cumulative release from 3D printed scaffolds of PCL/ F127 (70:30)/DEX-CYD and PCL/F127 (60:40)/DEX-CYD. (**B**) Individual release at each collection time point. (**C**) Cumulative release from PCL/ F68 (70:30)/DEX-CYD, and PCL/F68 (60:40)/DEX-CYD. (**D**) Individual release at each collection time point. All data represent mean ± SD (n=3).

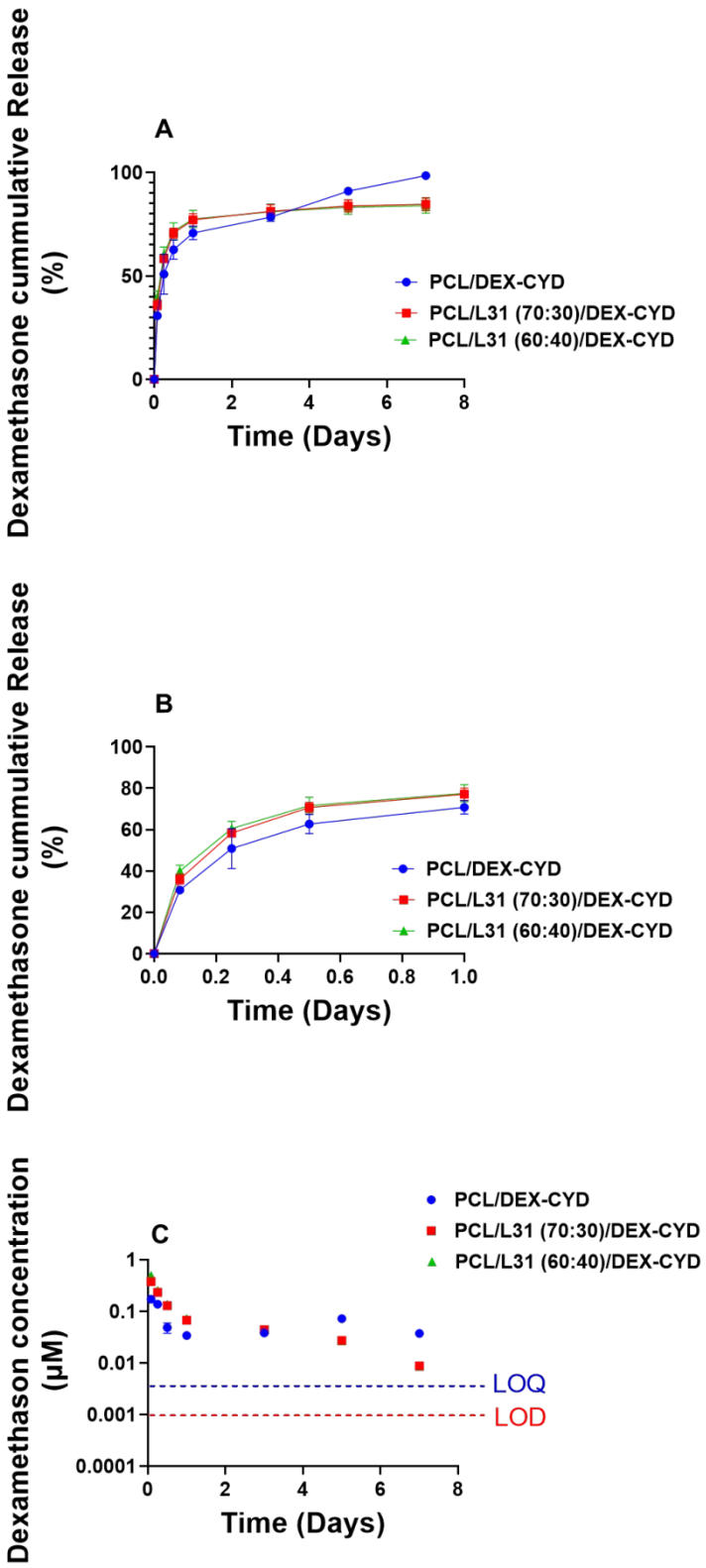

Figure S3: Cumulative and individual release at each collection time point from 3D printed scaffolds in 10mL PBS. (**A**) Cumulative release from 3D printed scaffolds of PCL, PCL/L31 (70:30), and PCL/L31 (60:40) loaded with 0.17% wt/wt of DEX-CYD. (**B**) Cumulative release over 1 day. (**C**) Individual release at each collection time point. Release medium was collected every two days after day 1. All data represent mean ± SD (n=3).

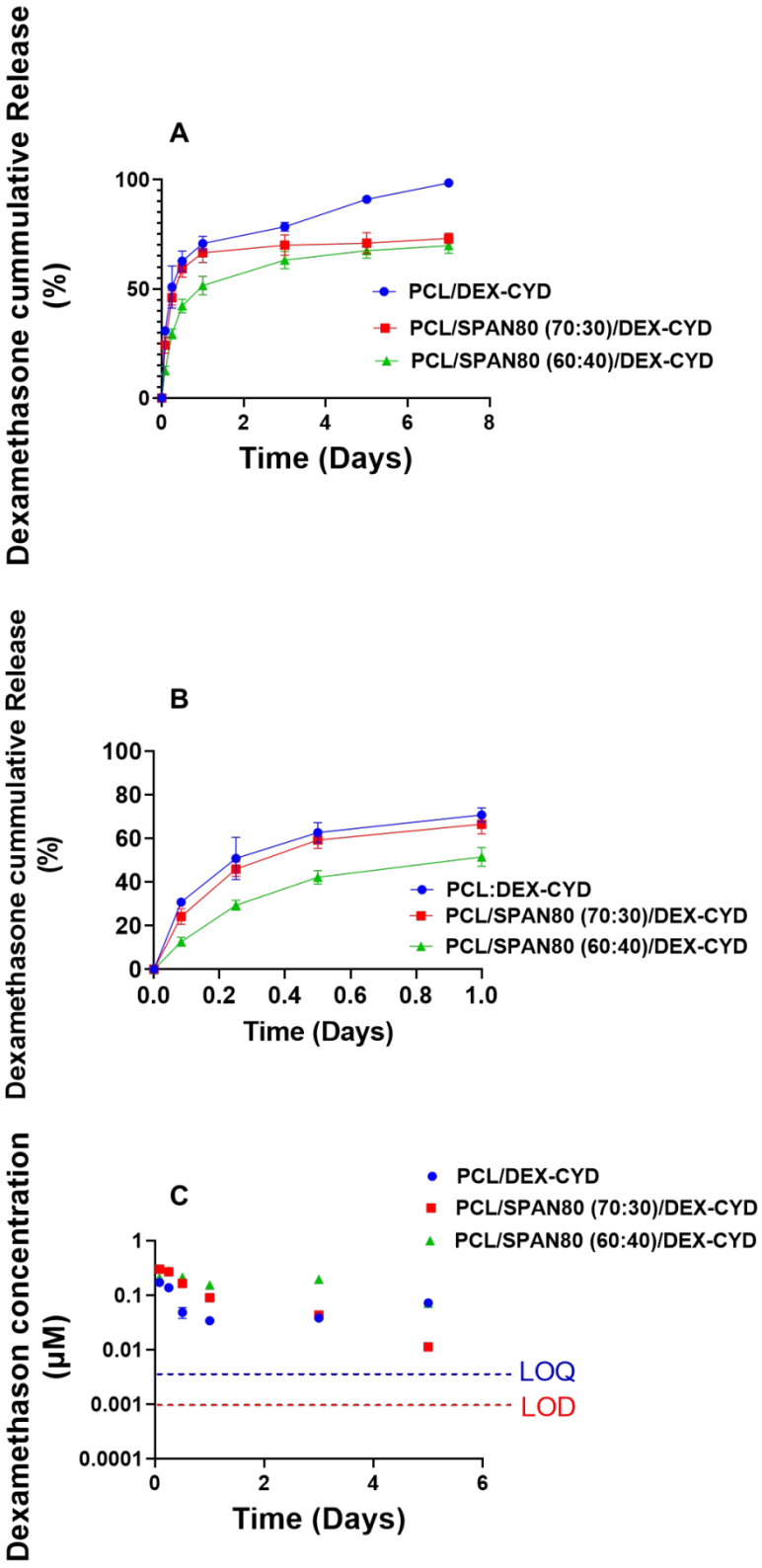

Figure S4: Cumulative and individual release at each collection time point from 3D printed scaffolds in 10mL PBS. (**A**) Cumulative release percentage of 3D printed scaffolds of PCL, PCL/Span80 (70:30), and PCL/Span80 (60:40) loaded with 0.17% wt/wt of DEX-CYD. (**B**) Cumulative release percentage over 1 day. (**C**) Individual release at each collection time point. Release medium was collected every two days after day 1. All data represent mean ± SD (n=3).

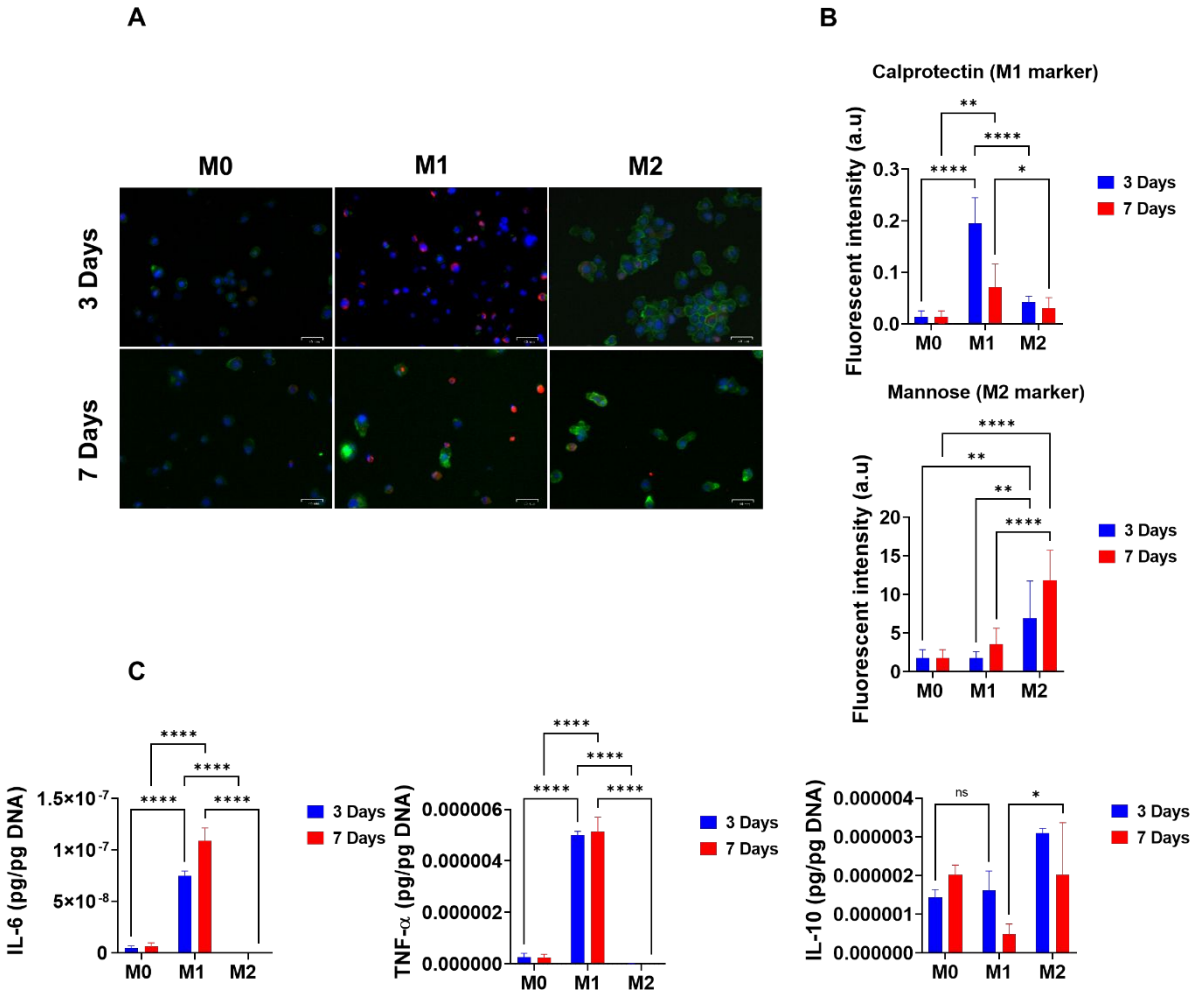

Figure S5: The polarisation of M0, M1 and M2 macrophages derived from THP-1 monocytes and M0, M1, and M2 cytokine expression. (**A**) Immunostaining of macrophages polarised at days 3 and 7 in RPMI single-media. The scale bar is 40 µm, respectively. (**B**) Fluorescence intensity was quantified by measuring the integrated density of Calprotectin (M1) and Mannose (M2) positive cells; this was normalized against the total number of cells (DAPI) by ImageJ. (**C**) pro-inflammatory cytokines TNF-α, IL-6, and anti-inflammatory IL-10 quantification of macrophages polarised in RPMI single media were quantified by ELISA and normalized to DNA content. All data represent mean ± SD (n=3). Statistical analysis was performed by one-way analysis of variance with Tukey's post hoc test indicated statistical differences with * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$, respectively.

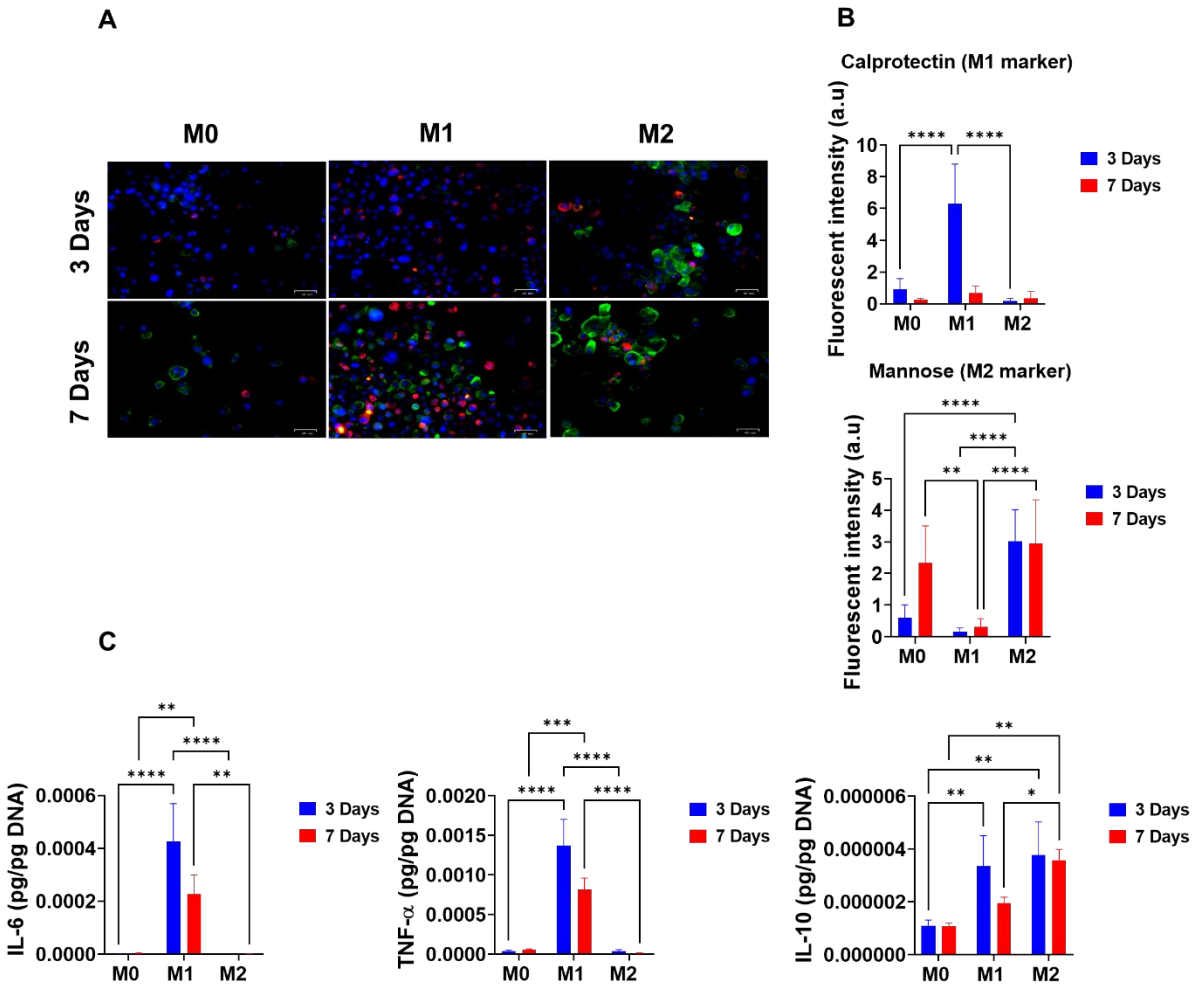

Figure S6: The polarisation of M0, M1 and M2 macrophages derived from THP-1 monocytes and M0, M1, and M2 cytokine expression. (**A**) Immunostaining of macrophages polarised at days 3 and 7 in a mixed-media (RPMI:α-MEM). The scale bar is 40 μm, respectively. (**B**) Fluorescence intensity was quantified by measuring the integrated density of Calprotectin (M1) and Mannose (M2) positive cells; this was normalized against the total number of cells (DAPI) by ImageJ. (**C**) Quantification of pro-inflammatory cytokines TNF-α, IL-6, and anti-inflammatory IL-10 from polarised macrophages in a mixed-medium (RPMI:α-MEM). All data represent mean ± SD (n=3). Statistical analysis was performed by one-way ANOVA with Tukey's post hoc. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$

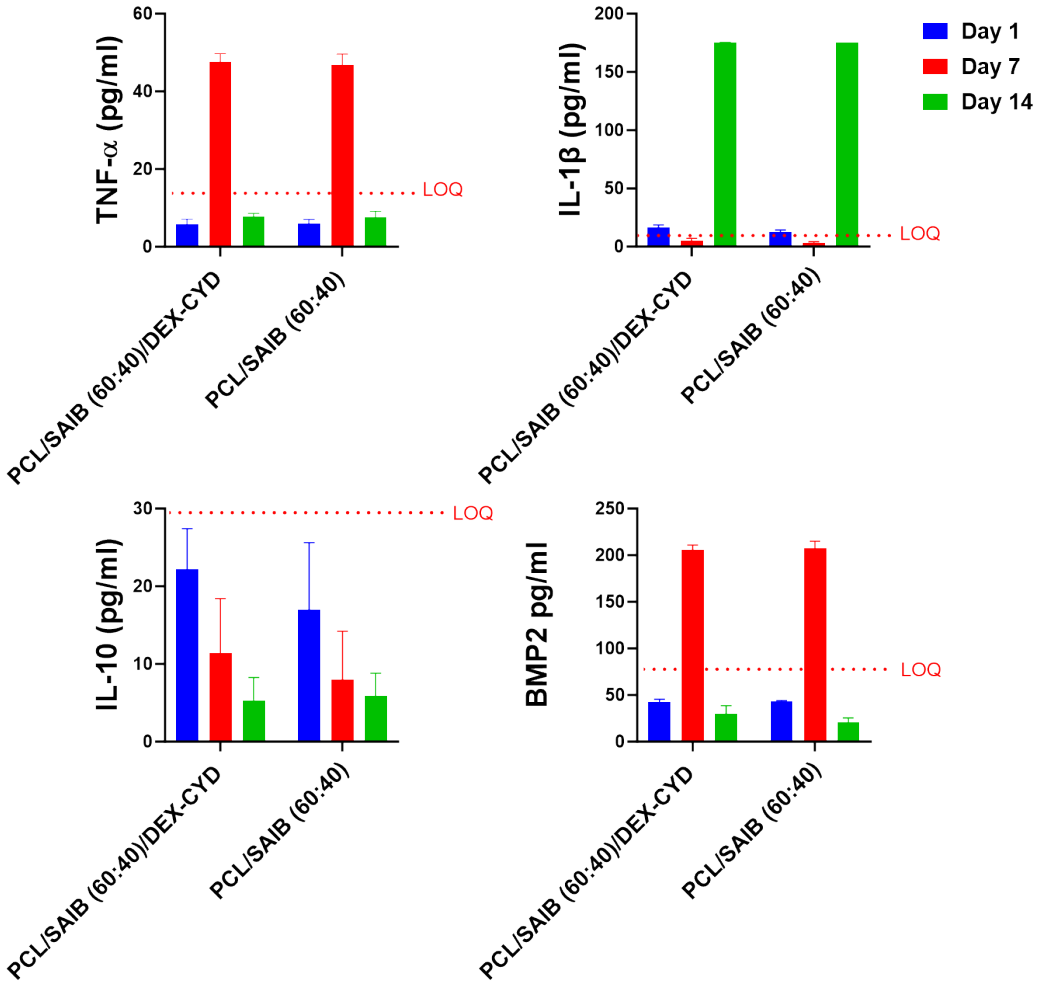

Figure S7. Quantification of cytokines from the macrophage-MSC co-culturing in scaffolds. Most values were below Limit of quantification (LOQ).

Table S2: (Figure 5 in the main text) One-way ANOVA Pairwise comparison MSC DNA in Co Culture media (RPMI:α-MEM 50:50) with or without of PCL/SAIB (60:40)/DEX-CYD scaffold at days 7,14, and 21.

MSC + Scaffold	Summary
7 Days vs. 14 Days	*
7 Days vs. 21 Days	***
14 Days vs. 21 Days	*
MSC Only	**Summary**
7 Days vs. 14 Days	ns
7 Days vs. 21 Days	ns
14 Days vs. 21 Days	ns

Table S3: (Figure 5 in the main text) One-way ANOVA Pairwise comparison of MSC DNA in RPMI with or without of PCL/SAIB (60:40)/DEX-CYD scaffold at days 7, 14, and 21.

MSC + Scaffold	Summary
7 Days vs. 14 Days	ns
7 Days vs. 21 Days	**
14 Days vs. 21 Days	**
MSC Only	**Summary**
7 Days vs. 14 Days	***
7 Days vs. 21 Days	****
14 Days vs. 21 Days	*

Table S4: (Figure 6 in the main text) One-way ANOVA Pairwise comparison of the effect of dexamethasone concentration on IL1β production from macrophage. THP-1 cells were first differentiated into M0 macrophages. LPS, GM-CSF and dexamethasone were added to the media (Except the M0 control) at days 3 and 7.

Samples	3 Days	7 Days
100 μM vs. 10μM	ns	ns
100 μM vs. 1 μM	ns	ns
100 μM vs. 0.1 μM	ns	ns
100 μM vs. 0.01 μM	ns	****
100 μM vs. 0.μM	ns	****
100 μM vs. M 0	****	**
10μM vs. 1 μM	ns	ns
10μM vs. 0.1 μM	ns	ns
10μM vs. 0.01 μM	ns	****
10μM vs. 0.μM	ns	****
10μM vs. M 0	****	*
1 μM vs. 0.1 μM	ns	ns
1 μM vs. 0.01 μM	ns	****
1 μM vs. 0.μM	ns	****
1 μM vs. M 0	****	ns
0.1 μM vs. 0.01 μM	ns	****
0.1 μM vs. 0.μM	ns	****
0.1 μM vs. M 0	****	**
0.01 μM vs. 0.μM	ns	****
0.01 μM vs. M 0	****	****
0.μM vs. M 0	****	****

Table S5: (Figure 6 in the main text) One-way ANOVA Pairwise comparison of the effect of dexamethasone concentration on IL-6 production from macrophage. THP-1 cells were first differentiated into M0 macrophages. LPS, GM-CSF and dexamethasone were added to the media (Except the M0 control) at days 3 and 7.

Samples	3 Days	7 Days
100 μM vs. 10μM	ns	ns
100 μM vs. 1 μM	ns	ns
100 μM vs. 0.1 μM	ns	ns
100 μM vs. 0.01 μM	*	*
100 μM vs. 0.μM	****	****
100 μM vs. M 0	***	ns
10μM vs. 1 μM	ns	ns
10μM vs. 0.1 μM	ns	ns
10μM vs. 0.01 μM	**	*
10μM vs. 0.μM	****	****
10μM vs. M 0	**	ns
1 μM vs. 0.1 μM	ns	ns
1 μM vs. 0.01 μM	**	**
1 μM vs. 0.μM	****	****
1 μM vs. M 0	**	ns
0.1 μM vs. 0.01 μM	ns	*
0.1 μM vs. 0.μM	****	****
0.1 μM vs. M 0	****	ns
0.01 μM vs. 0.μM	****	****
0.01 μM vs. M 0	****	***
0.μM vs. M 0	****	****

Table S6: (Figure 6 in the main text) One-way ANOVA Pairwise comparison of the effect of dexamethasone concentration on TNF-α production from macrophage. THP-1 cells were first differentiated into M0 macrophages. LPS, GM-CSF and dexamethasone were added to the media (Except the M0 control) at days 3 and 7.

Samples	3 Days	7 Days
100 μM vs. 10μM	ns	ns
100 μM vs. 1 μM	ns	*
100 μM vs. 0.1 μM	ns	*
100 μM vs. 0.01 μM	ns	ns
100 μM vs. 0.μM	**	ns
100 μM vs. M 0	ns	****
10μM vs. 1 μM	ns	ns
10μM vs. 0.1 μM	ns	ns
10μM vs. 0.01 μM	ns	ns
10μM vs. 0.μM	**	ns
10μM vs. M 0	ns	***
1 μM vs. 0.1 μM	ns	ns

1 μM vs. 0.01 μM	ns	ns
1 μM vs. 0.μM	**	***
1 μM vs. M 0	ns	ns
0.1 μM vs. 0.01 μM	ns	ns
0.1 μM vs. 0.μM	**	***
0.1 μM vs. M 0	ns	*
0.01 μM vs. 0.μM	ns	**
0.01 μM vs. M 0	*	**
0.μM vs. M 0	***	****

Table S7: (Figure 7 in the main text) One-way ANOVA Pairwise comparison of the effect of dexamethasone concentration on IL-6 production from macrophage. THP-1 cells were first differentiated into M0 macrophages. LPS, GM-CSF and PCL/DEX-CYD or PCL/SAIB (60:40)/DEX-CYD were added to the media (Except the M0 control) at days 3 and 7.

Samples	3 Days	7 Days
PCL/DEX-CYD vs. PCL/SAIB (60:40)/DEX-CYD	**	**
PCL/DEX-CYD vs. PCL	ns	ns
PCL/DEX-CYD vs. M0	*	****
PCL/SAIB (60:40)/DEX-CYD vs. PCL	ns	ns
PCL/SAIB (60:40)/DEX-CYD vs. M0	ns	ns
PCL vs. M0	ns	ns

Table S8: (Figure 7 in the main text) One-way ANOVA Pairwise comparison of the effect of dexamethasone concentration on TNF-α production from macrophage. THP-1 cells were first differentiated into M0 macrophages. LPS, GM-CSF and PCL/DEX-CYD or PCL/SAIB (60:40)/DEX-CYD were added to the media (Except the M0 control) at days 3 and 7.

samples	3 Days	7 Days
PCL/DEX-CYD vs. PCL/SAIB (60:40)/DEX-CYD	ns	****
PCL/DEX-CYD vs. PCL	ns	***
PCL/DEX-CYD vs. M0	*	****
PCL/SAIB (60:40)/DEX-CYD vs. PCL	ns	****
PCL/SAIB (60:40)/DEX-CYD vs. M0	**	ns
PCL vs. M0	ns	****

Table S9: **(**For figure 8 in main text): One-way ANOVA Pairwise comparison for ALP production in MSCs co-cultured with M1 macrophages in the presence of 3D printed PCL/SAIB (60:40)/DEX-CYD scaffolds over 7, 14, and 21 days.

Samples	7 Days	14 Days	21 Days
MSC + Scaffold vs. MSC only	****	****	****

MSC + Scaffold vs. M1+MSC + Scaffold	ns	****	ns
MSC + Scaffold vs. M1+MSC	*	****	****
MSC + Scaffold vs. M0+MSC	ns	****	****
MSC only vs. M1+MSC + Scaffold	***	****	****
MSC only vs. M1+MSC	**	*	ns
MSC only vs. M0+MSC	***	ns	ns
M1+MSC + Scaffold vs. M1+MSC	ns	****	****
M1+MSC + Scaffold vs. M0+MSC	ns	****	****
M1+MSC vs. M0+MSC	ns	ns	ns

Table S10: (For figure 8 in main text): One-way ANOVA Pairwise comparison for Alizarin Red production in MSCs co-cultured with M1 macrophages in the presence of 3D printed PCL/SAIB (60:40)/DEX-CYD scaffolds over 7, 14, and 21 days.

Samples	7 Days	14 Days	21 Days
MSC + Scaffold vs. MSC only	**	ns	***
MSC + Scaffold vs. M1+MSC + Scaffold	***	ns	ns
MSC + Scaffold vs. M1+MSC	ns	ns	**
MSC + Scaffold vs. M0+MSC	ns	ns	***
MSC only vs. M1+MSC + Scaffold	****	ns	**
MSC only vs. M1+MSC	*	ns	ns
MSC only vs. M0+MSC	**	ns	ns
M1+MSC + Scaffold vs. M1+MSC	***	ns	**
M1+MSC + Scaffold vs. M0+MSC	**	ns	**
M1+MSC vs. M0+MSC	ns	ns	ns

Table S11: (For figure 8 in main text): One-way ANOVA Pairwise comparison for Bone morphogenic protein-2 (BMP-2) production in MSCs co-cultured with M1 macrophages in the presence of 3D printed PCL/SAIB (60:40)/DEX-CYD scaffolds over 7, 14, and 21 days.

Samples	7 Days	21 Days
MSC + Scaffold vs. MSC only	****	****
MSC + Scaffold vs. M1+MSC + Scaffold	*	**
MSC + Scaffold vs. M1+MSC	ns	****
MSC + Scaffold vs. M0+MSC	*	****
MSC only vs. M1+MSC + Scaffold	****	****
MSC only vs. M1+MSC	****	***
MSC only vs. M0+MSC	**	ns
M1+MSC + Scaffold vs. M1+MSC	*	****
M1+MSC + Scaffold vs. M0+MSC	****	****
M1+MSC vs. M0+MSC	****	****

Table S12 : (For figure 8 in main text): One-way ANOVA Pairwise comparison for RUNX2 gene expression in MSCs co-cultured with M1 macrophages in the presence of 3D printed PCL/SAIB (60:40)/DEX-CYD scaffolds over 3, 7, and 21 days.

Samples	3 Days	7 Days	21 Days
MSC only vs. MSC+ Scaffold	*	**	*
MSC only vs. M1+MSC + Scaffold	**	****	ns
MSC only vs. M1+MSC	*	ns	ns
MSC only vs. M0+MSC	ns	ns	ns
MSC+ Scaffold vs. M1+MSC + Scaffold	ns	**	ns
MSC+ Scaffold vs. M1+MSC	ns	ns	*
MSC+ Scaffold vs. M0+MSC	*	ns	ns
M1+MSC + Scaffold vs. M1+MSC	ns	**	ns
M1+MSC + Scaffold vs. M0+MSC	**	***	ns
M1+MSC vs. M0+MSC	*	ns	ns

Table S13: (For figure 9 in main text) One-way ANOVA Pairwise comparison for IL-6 production in MSCs co-cultured with M1 macrophages in the presence of 3D printed PCL/SAIB (60:40)/DEX-CYD scaffolds over 3, 7, 14, and 21 days.

Samples	3 Days	7Days	14 Days	21 Days
M1+MSC+Scaffold vs. M1+MSC	****	****	****	****
M1+MSC+Scaffold vs. M0+ MSC	****	**	****	***
M1+MSC+Scaffold vs. MSC only	ns	ns	***	***
M1+MSC+Scaffold vs. M0	ns	ns	ns	ns
M1+MSC vs. M0+ MSC	***	****	**	***
M1+MSC vs. MSC only	****	****	****	***
M1+MSC vs. M0	****	****	****	****
M0+ MSC vs. MSC only	****	**	ns	ns
M0+ MSC vs. M0	****	***	****	***
MSC only vs. M0	ns	ns	***	***

Table S14: (For figure 9 in main text) One-way ANOVA Pairwise comparison for TGF-β1 production in MSCs co-cultured with M1 macrophages in the presence of 3D printed PCL/SAIB (60:40)/DEX-CYD scaffolds over 3, 7, 14, and 21 days.

Samples	3 Days	7 Days	14 Days	21 Days
M1+MSC+Scaffold vs. M1+MSC	ns	ns	***	****
M1+MSC+Scaffold vs. M0+ MSC	ns	ns	***	***
M1+MSC+Scaffold vs. MSC only	ns	ns	**	****
M1+MSC+Scaffold vs. M0	ns	ns	ns	ns
M1+MSC vs. M0+ MSC	ns	ns	ns	ns
M1+MSC vs. MSC only	ns	ns	ns	**
M1+MSC vs. M0	ns	ns	***	****
M0+ MSC vs. MSC only	ns	ns	ns	**
M0+ MSC vs. M0	ns	ns	***	****
MSC only vs. M0	ns	ns	**	****

Table S15: (For figure 9 in main text) One-way ANOVA Pairwise comparison for IL-10 production in MSCs co-cultured with M1 macrophages in the presence of 3D printed PCL/SAIB (60:40)/DEX-CYD scaffolds over 3, 7, 14, and 21 days.

Samples	3 Days	7 Days	14 Days	21 Days
M1+MSC+Scaffold vs. M1+MSC	ns	ns	ns	ns
M1+MSC+Scaffold vs. M0+ MSC	ns	ns	**	ns
M1+MSC+Scaffold vs. MSC only	***	ns	ns	ns
M1+MSC+Scaffold vs. M0	***	ns	ns	ns
M1+MSC vs. M0+ MSC	ns	ns	*	ns
M1+MSC vs. MSC only	*	ns	ns	ns
M1+MSC vs. M0	**	ns	ns	ns
M0+ MSC vs. MSC only	***	ns	*	ns
M0+ MSC vs. M0	***	ns	*	ns
MSC only vs. M0	ns	ns	ns	ns