

RESEARCH ARTICLE

3D printing biocompatible materials with Multi Jet Fusion for bioreactor applications

Supplementary File



Figure S1. Schematic illustration showing the experimental design for long-term sub-culture of L929 fibroblasts and MC3T3e1 osteoblasts cultured on PA-12 cell culture chambers and positive control (plate) sub-cultured from uncoated, PDL and CLG-coated PA-12 cell culture chambers.

		L929 Fi	ibroblasts		MC3T3e1 Osteoblasts			
	l Norr	nal medium	Leachate		Normal medium		Leachate	
	No cell	No cell With cell		No cell With cell		No cell With cell		۱ With cell
	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst
Day 2	Phalloidin	Phalloidin	Phalloidin	Phalloidin	Phalloidin	Phalloidin	Phalloidin	Phalloidin
	Merged	Merged	Merged	Merged	Merged	Merged	Merged	Merged

Figure S2. Representative fluorescence microscopy images of L929 Fibroblasts and MC3T3e1 osteoblasts cultured using normal and respective leachate medium showing cell attachment and increase in cell density following culture on polystyrene plates after 2 days.

	Dav	y 2		Day 4				
l Positive Ctrl (uncoated) no cell	Positive Ctrl (uncoated) with cell	PA-12 (uncoated) no cell	PA-12 (uncoated) with cell	Positive Ctrl (uncoated) no cell	Positive Ctrl (uncoated) with cell	PA-12 (uncoated) no cell	PA-12 (uncoated) with cell	
Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	
Phalloidin	Phalloidin	Phalloidin	Phalloidin	Phalloidin	Phalloidin	Phalloidin	Phalloidin	
Merged	Merged	Merged	Merged	Merged	Merged	Merged	Mergeds	

Figure S3. Representative fluorescence microscopy images of L929 fibroblasts cultured on uncoated 3D-printed PA-12 and positive control (plate) cell culture chambers after day 2 and 4, respectively.

2	D	ay 2		Day 4				
Positive Ctrl (plasma) no cell	Positive Ctrl (plasma) with cell	PA-12 (plasma) no cell	PA-12 (plasma) with cell	Positive Ctrl (plasma) no cell	Positive Ctrl (plasma) with cell	PA-12 (plasma) no cell	PA-12 (plasma) with cell	
Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	
Phalloidin	Phalloidin to the second	Phalloidin	Phalloidin	Phalloidin	Phalloidin	Phalloidin	Phalloidin	
Merged	Merged	Merged	Merged	Merged	Merged	Merged	Merged	

Figure S4. Representative fluorescence microscopy images of L929 fibroblasts cultured on O₂ plasma-treated 3D-printed PA-12 and positive control (plate) cell culture chambers after day 2 and 4, respectively.



Figure S5. Representative fluorescence microscopy images of L929 fibroblasts cultured on PDL-coated 3D-printed PA-12 and positive control (plate) cell culture chambers after 2 days.



Figure S6. Representative fluorescence microscopy images of L929 fibroblasts cultured on CLG-coated 3D-printed PA-12 and positive control (plate) cell culture chambers after day 2 and 4, respectively.

		Day 2		·	Day 4			
Positive Ctrl (uncoated) no cell	Positive Ctrl (uncoated) with cell	PA-12 (uncoated) no cell	PA-12 (uncoated) with cell	Positive Ctrl (uncoated) no cell	Positive Ctrl (uncoated) with cell	PA-12 (uncoated) no cell	PA-12 (uncoated) with cell	
Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	
Phalloidin	Phalloidin .	Phalloidin	Phalloidin	Phalloidin	Phalloidin	Phalloidin	Phalloidin	
Merged	Merged	Merged	Merged	Merged	Merged	Merged	Merged	

Figure S7. Representative fluorescence microscopy images of MC3T3e1 osteoblasts cultured on uncoated 3D-printed PA-12 and positive control (plate) cell culture chambers after day 2 and 4, respectively.



Figure S8. Representative fluorescence microscopy images of MC3T3e1 osteoblasts cultured on O_2 plasma-treated 3D-printed PA-12 and positive control (plate) cell culture chambers after day 2 and 4, respectively.



Figure S9. Representative fluorescence microscopy images of MC3T3e1 osteoblasts cultured on PDL-coated 3D-printed PA-12 and positive control (plate) cell culture chambers after 2 days.



Figure S10. Representative fluorescence microscopy images of MC3T3e1 osteoblasts cultured on CLG-coated 3D-printed PA-12 and positive control (plate) cell culture chambers after day 2 and 4, respectively.

A (i) No cells + uncoated PA-12 1810 X388 - 560m 27 27 561	(ii) No cells +PDL-coated PA-12 1840 X380 559m 27 27 SEI	(iii) No cells +CLG-coated PA-12
B (1) No cells + Uncoated PA-12	(ii) No cells +PDL-coated PA-12 No. 10kU Xabe 560m 27 27 SED	(iii) No cells +CLG-coated PA-12
C (i) No cells +uncoated	(ii) No cells +PDL-coated	(iii) No cells +CLG-coated
10kU X300 50.000 000 30 27 SE1	polystyrene 19к0 ×300 <u>30мм</u> 0800 30 27 SEI	10KU X300 50mm 0000 34 27 SEI

Figure S11. Representative scanning electron microscopy images showing the (i) uncoated, (ii) PDL-coated and (iii) CLG-coated surface morphology of PA-12 cell culture chambers in (A) DMEM medium without cells and (B) aMEM medium without cells are shown for reference. Representative scanning electron microscopy images showing the surface morphology of (i) uncoated, (ii) PDL-coated and (iii) CLG-coated polystyrene cell culture chambers in (C) DMEM medium without cells are shown for reference.

	Positive Ctrl (uncoated) no cell	Positive Ctrl (uncoated) with cell	PA-12 (uncoated) no cell	PA-12 (uncoated) with cell	Positive Ctrl (CLG) no cell	Positive Ctrl (CLG) with cell	PA-12 (CLG) no cell	PA-12 (CLG) with cell
roblasts	Day 2	-Day 2	Day 2	Day 2	Day 2	Day 2	Day 2	Day 2 ∞er
L929 Fib	Day 4	Day 4	Day 4	Day 4	Day 4	Day 4	Day 4	Day 4
steoblasts	Day 2	Day 2	Day 2	Day 2	Day 2	Day 2	Day 2	Day 2
MC3T3e1 C	Day 4	Day 4	Day 4	Day 4	Day 4	Day 4	Day 4	Day 4

Figure S12. Representative fluorescence microscopy images of L929 fibroblasts and MC3T3e1 osteoblasts stained for glutathione evaluation following culture on uncoated and CLG-coated 3D-printed PA-12 and positive control (plate) cell culture chambers after day 2 and day 4, respectively.

	Da	ay 2		Day 4				
l Positive Ctrl (uncoated) no cell	Positive Ctrl (uncoated) with cell	PA-12 (uncoated) no cell	PA-12 (uncoated) with cell	Positive Ctrl (uncoated) no cell	Positive Ctrl (uncoated) with cell	PA-12 (uncoated) no cell	PA-12 (uncoated) with cell	
Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	
Anti-ki67	Anti-ki67	Anti-ki67	Anti-ki67	Anti-ki67	Anti-ki67	Anti-ki67	Anti-ki67	
Anti-p53	Anti-p53	Anti-p53	Anti-p53	Anti-p53	Anti-p53	Anti-p53	Anti-p53	
Merged	Merged	Merged	Merged	Merged	Merged 2007	Merged	Merged	

Figure S13. Representative fluorescence microscopy images of L929 Fibroblasts immunostained to determine the expression of ki67 and p53 following culture on uncoated 3D-printed PA-12 and positive control (plate) cell culture chambers after day 2 and day 4, respectively.



Figure S14. Representative fluorescence microscopy images of L929 Fibroblasts immunostained to determine the expression of ki67 and p53 following culture on PDL-coated 3D-printed PA-12 and positive control (plate) cell culture chambers after 2 days.

		Day 2		Day 4				
Positive Ctrl (CLG) no cell	Positive Ctrl (CLG) with cell	PA-12 (CLG) no cell	PA-12 (CLG) with cell	Positive Ctrl (CLG) no cell	Positive Ctrl (CLG) with cell	PA-12 (CLG) no cell	PA-12 (CLG) with cell	
Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	
Anti-ki67	Anti-ki67	Anti-ki67	Anti-ki67	Anti-ki67	Anti-ki67	Anti-ki67	Anti-ki67	
Anti-p53	Anti-p53	Anti-p53	Anti-p53	Anti-p53	Anti-p53	Anti-p53	Anti-p53	
Merged	Merged	Merged	Merged	Merged	Merged	Merged	Merged	

Figure S15. Representative fluorescence microscopy images of L929 Fibroblasts immunostained to determine the expression of ki67 and p53 following culture on CLG-coated 3D-printed PA-12 and positive control (plate) cell culture chambers after day 2 and day 4, respectively.

-	Da	ay 2		Day 4				
l Positive Ctrl (uncoated) no cell	Positive Ctrl (uncoated) with cell	PA-12 (uncoated) no cell	PA-12 (uncoated) with cell	lí Positive Ctrl (uncoated) no cell	Positive Ctrl (uncoated) with cell	PA-12 (uncoated) no cell	PA-12 (uncoated) with cell	
Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	
Anti-ki67	Anti-ki67	Anti-ki67	Anti-ki67	Anti-ki67	Anti-ki67	Anti-ki67	Anti-ki67	
Anti-p53	Anti-p53	Anti-p53	Anti-p53	Anti-p53	Anti-p53	Anti-p53	Anti-p53	
Merged _{عو} یہ	Merged	Merged	Merged	Merged	Merged	Merged	Merged	

Figure \$16. Representative fluorescence microscopy images of MC3T3e1 osteoblasts immunostained to determine the expression of ki67 and p53 following culture on uncoated 3D-printed PA-12 and positive control (plate) cell culture chambers after day 2 and day 4, respectively.



Figure \$17. Representative fluorescence microscopy images of MC3T3e1 osteoblasts immunostained to determine the expression of ki67 and p53 following culture on PDL-coated 3D-printed PA-12 and positive control (plate) cell culture chambers after 2 days.

	C	Day 2		Day 4				
l Positive Ctrl (CLG) no cell	Positive Ctrl (CLG) with cell	PA-12 (CLG) no cell	PA-12 (CLG) with cell	II Positive Ctrl (CLG) no cell	Positive Ctrl (CLG) with cell	PA-12 (CLG) no cell	ا PA-12 (CLG) with cell	
Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	
Anti-ki67	Anti-ki67	Anti-ki67	Anti-ki67	Anti-ki67	Anti-ki67	Anti-ki67	Anti-ki67	
Anti-p53	Anti-p53	Anti-p53	Anti-p53	Anti-p53	Anti-p53	Anti-p53	Anti-p53	
Merged	Merged	Merged	Merged	Merged	Merged	Merged	Merged	

Figure S18. Representative fluorescence microscopy images of MC3T3e1 osteoblasts immunostained to determine the expression of ki67 and p53 following culture on CLG-coated 3D-printed PA-12 and positive control (plate) cell culture chambers after day 2 and day 4, respectively.







Figure S20. Representative fluorescence images showing the long-term viability of L929 fibroblasts sub-cultured on uncoated 3D-printed PA-12 and positive control (plate) cell culture chambers following long-term culture after day 8 and day 12, respectively.



Figure S21. Representative fluorescence images showing the long-term viability of L929 fibroblasts sub-cultured on PDL-coated 3D-printed PA-12 and positive control (plate) cell culture chambers following long-term culture after day 8, respectively.



Figure S22. Representative fluorescence images showing the long-term viability of L929 fibroblasts sub-cultured on CLG-coated 3D-printed PA-12 and positive control (plate) cell culture chambers following long-term culture after day 8 and day 12, respectively.



Figure S23. Representative fluorescence images showing the long-term viability of MC3T3e1 osteoblasts sub-cultured on uncoated 3D-printed PA-12 and positive control (plate) cell culture chambers following long-term culture after day 8 and day 12, respectively.

	Positive Ctrl (plate to plate) (PDL) no cell	Positive Ctrl (plate to plate) (PDL) with cell	PA-12 to PA-12 (PDL) no cell	PA-12 to PA-12 (PDL) with cell	PA-12 to Plate (PDL) no cell	PA-12 to Plate (PDL) with cell
	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst	Hoechst
Day 8	Phalloidin	Phalloidin	Phalloidin	Phalloidin	Phalloidin	Phalloidin
	Merged	Merged	Merged	Merged	Merged	Merged

Figure S24. Representative fluorescence images showing the long-term viability of MC3T3e1 osteoblasts sub-cultured on PDL-coated 3D-printed PA-12 and positive control (plate) cell culture chambers following long-term culture after day 8, respectively.



Figure S25. Representative fluorescence images showing the long-term viability of MC3T3e1 osteoblasts sub-cultured on CLG-coated 3D-printed PA-12 and positive control (plate) cell culture chambers following long-term culture after day 8 and day 12, respectively.