**Janus 3D printed dynamic scaffolds for nanodeflection-driven bone regeneration**

Sandra Camarero-Espinosa1 and Lorenzo Moroni1\*.

*1MERLN Institute for Technology-inspired Regenerative Medicine, Complex Tissue Regeneration Department, Maastricht University, P.O. Box 616, 6200MD Maastricht, The Netherlands*

***Supplementary Information***

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Figure S1. Scanning Electron Microscopy (SEM) images of as-extruded filaments of PLA:PCL blends where an initial phase-segregation is observed. PLA concentration increases from left to right. White scale bars are 500 µm, green are 100 µm, red are 50 µm and black are 20 µm.

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**Figure S2.** a) Polarized optical microscopy images of PLA (top), 50:50 PLA:PCL blend (middle) and PCL (bottom) upon cooling from 200°C melts showing the crystallization of PCL in the neat state and mixed with PLA. Scale bars are 100µm, red scale bars are 10 µm. b) X-ray diffraction spectra of PLA, PCL and PLA:PCL blends at 75:25, 50:50 and 25:75 ratios showing a progressive increase on the intensity of the crystallization peaks of PCL (110 and 200) and a characteristic amorphous hallo of PLA.



Figure S3. Analysis of the area (a) and aspect ratio (b) of the phase segregation on the cross-sectional direction for PLA:PCL ratios of 20:80, 30:70, 40:60, 60:40, 70:30 and 80:20. Bars show median and 95% CI. Statistical significance was calculated from 1-way ANOVA. (\*\*\*\*) p<0.0001, (\*\*\*) p<0.0002, (\*\*) p<0.0021 and (\*) p<0.0332.



Figure S4. Analysis of the area (a) and aspect ratio (b) of the phase segregation on the longitudinal direction of the scaffold fibers for PLA:PCL ratios of 20:80, 30:70, 40:60, 60:40, 70:30 and 80:20. Bars show median and 95% CI. Statistical significance was calculated from 1-way ANOVA. (\*\*\*\*) p<0.0001, (\*\*\*) p<0.0002, (\*\*) p<0.0021 and (\*) p<0.0332.



Figure S5. Phase segregation of PLA:PCL blends labelled with FITC (488, green) and Rhodamine B (580, pink), respectively, on the cross-section of additive manufactured fibers as visualized by Light Scanning Microscopy (LSM). PLA concentration increases from left to right images. At 50:50 PLA:PCL composition a clear Janus phase segregation is observed. Scale bars are 100 µm.



Figure S6. Representative ultrasound waves generated to stimulate the scaffolds as detected with a needle hydrophone in liquid media after crossing the same polystyrene layer (Petri-dish) used in experiments with scaffolds, showing a decrease of the frequency from top to bottom.



Figure S7. Fast Fourier Transform (FFT) of the ultrasound waves used to stimulate the scaffolds showing (from top to bottom) an increase on the frequency.



Figure S8. Representative load-displacement traces of PCL, PLA and Janus scaffolds measured under 3-point bending with a 40N load cell at a 0,01mm/s deformation rate.

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Figure S9. Representative ultrasound waves transmitted after crossing a polystyrene layer (Petri-dish) and the different scaffold compositions (Janus, PCL and PLA), measured with a needle hydrophone in liquid media. Scaffolds were excited with an ultrasound wave of 38 kHz.



Figure S10. Fast Fourier Transform (FFT) of the ultrasound transmitted through the scaffolds showing (from top to bottom) an increase on the frequency of the first peak corresponding to the scaffolds transmitted wave, and a secondary one corresponding to the emitted wave (38 kHz).



Figure S11. Fluorescent light microscopy images of BMSCs cultured for 1 week on Janus, PCL and PLA scaffolds and stimulated 30 minutes daily at 40kHz showing an increased fibronectin deposition on Janus scaffolds. Cells were stained for F-actin (green), DNA (blue) and fibronectin (red). Scale bar is 200 µm.

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Figure S12. Osteocalcin release normalized to total DNA content after 3 weeks of osteogenic differentiation of BMSC cultured on Janus, PCL and PLA scaffolds under stimulated (+US) and static (-US) conditions, showing an ultrasound dependent release for cells cultured on Janus and PLA scaffolds that was higher for Janus than PCL scaffolds.

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Figure S13. Alkaline phosphatase release normalized to total DNA content after 3 weeks of osteogenic differentiation of BMSC cultured on Janus, PCL and PLA scaffolds under stimulated (+US) and static (-US) conditions, showing an ultrasound-independent release that is maximum for cells cultured on Janus scaffolds.



Figure S14. Fluorescent light microscopy images of BMSCs cultured for 3 weeks on Janus, PCL and PLA scaffolds in osteogenic media and stimulated 30 minutes daily at 40kHz, showing the expression of dihydropyridine receptor (DHPR, a voltage gated Ca+2 ion channel) on Janus scaffolds. Cells were stained for F-actin (green), DNA (blue) and DHPR (red). Scale bar is 200 µm.

Table S1. Measured frequency and amplitude of the different generated ultrasound waves. Set-ups 2, 6 and 10 were used for cell work and are referred as 10, 25 and 40 kHz.

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| Set-up | Frequency (kHz) | Intensity (mV) |
| 2 | 11,7 | 0,3 |
| 4 | 13,6 | 0,3 |
| 6 | 22,2 | 0,3 |
| 8 | 25,5 | 0,3 |
| 10 | 38,7 | 0,4 |

Table S2. Flexural modulus (MPa) calculated for the different scaffolds compositions; PLA, PCL and Janus.

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| Material | Flexural modulus (MPa) |
| PLA | 2326 ± 36 |
| PCL | 303 ± 70 |
| Janus | 759 ± 62 |
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Table S3. Measured frequencies and intensities of the ultrasound waves transmitted by the scaffolds.

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| Material | Frequency | Intensity (mV) |
| PCL | 5,4 | 0,08 |
|  | 36,8 | 0,07 |
| PLA | 17,3 | 0,06 |
|  | 38,9 | 0,08 |
| Janus | 15,4 | 0,05 |
|  | 38,5 | 0,1 |