Ultrasound-activated piezoelectric PLLA:PLCL films for dual modulation of microglial polarization

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ABSTRACT

Optimized **PLLA:PLCL** blend

US stimulation → Processability ↑, Flexibility ↑, Faster bioresorption → Piezoelectricity driven microglial activation

M1-like and M2-like terminology is used here as a simplified operational framework to describe dominant polarization trends [1].

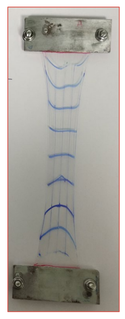
Microglia (resting)

M1 (pro-inflammatory) — CD40, CD16
- Anti-tumor immunity
- Pathogen clearance
- Inflammation modeling

M2 (anti-inflammatory) — CD206, CD163, Arg-1
- Tissue regeneration
- Anti-inflammatory response
- Biomaterial integration

METHODS

Material fabrication



DR1: As-made film

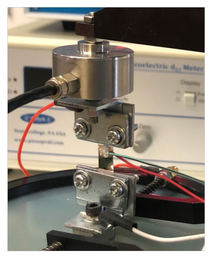
DR4: 400% stretched

DR4AN80: Stretched + annealed (80 ºC)

Composition (PLLA:PLCL): PLLA, 90:10, 80:20, 70:30, 60:40

Incubation: Day-0 & day-30 in PBS at 37 ºC

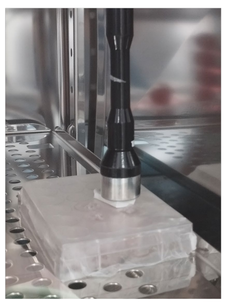
Material characterization



Piezoelectric response

Functional properties
- DSC
- Tensile test
- TGA
- PRS
- 2D-WAXS

Cell culture & US stimulation



HMC-3 cells
Pulsed US

Evaluation of the effect on microglia

RESULTS

Degree of crystallinity

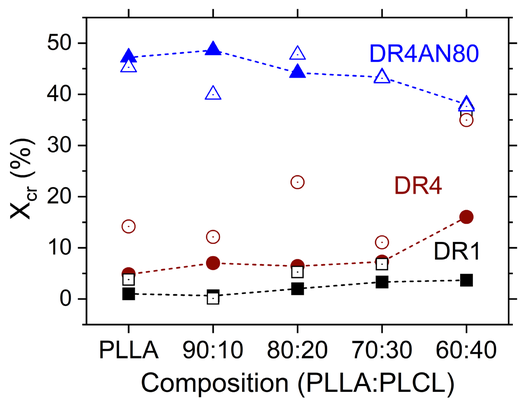


Figure 1. Degree of crystallinity calculated from Differential Scanning Calorimetry (DSC) curves. **DR4 films** remain **nearly-amorphous,** but **the degree of crystallinity increases during the incubation of the samples in PBS at 37 ºC.**

**Solid-symbol: 0 d*
Open-symbol: 30 d

Mechanical properties

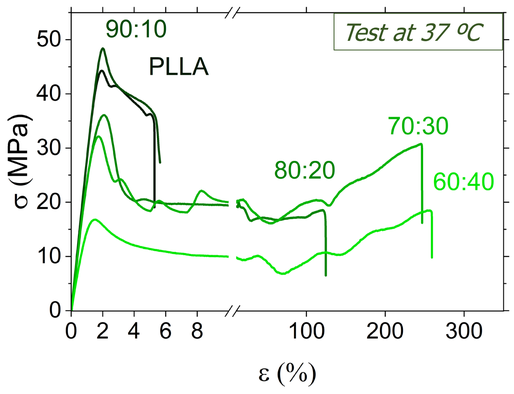


Figure 2. Tensile test curves. **PLCL improves ductility and toughness.**

Thermal stability

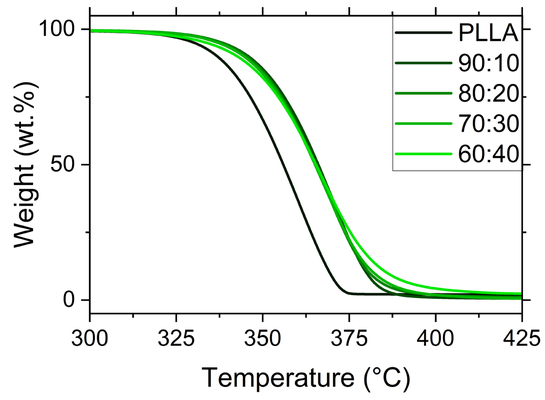


Figure 3. Thermogravimetric Analysis (TGA) curves. **PLCL increases thermal stability.**

Macromolecular orientation

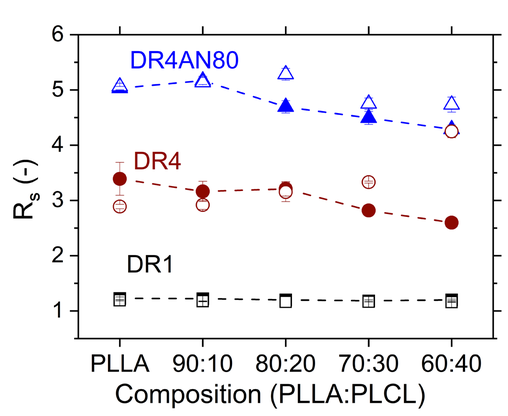


Figure 4. Orientation factor calculated from Polarized Raman Spectroscopy (PRS). **Oriented crystal regions were generated by annealing.**

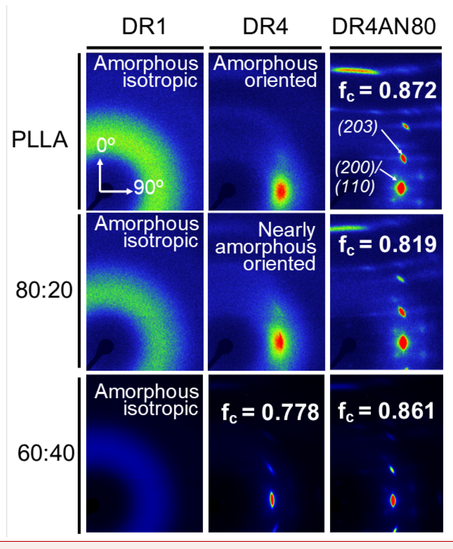


Figure 5. 2D-WAXS patterns were used to calculate Herman´s orientation function (f_c) values. **PLCL content does not compromise orientation and favors the formation of oriented crystal domains, specially in 60:40 PLLA:PLCL blends.**

Piezoelectric response

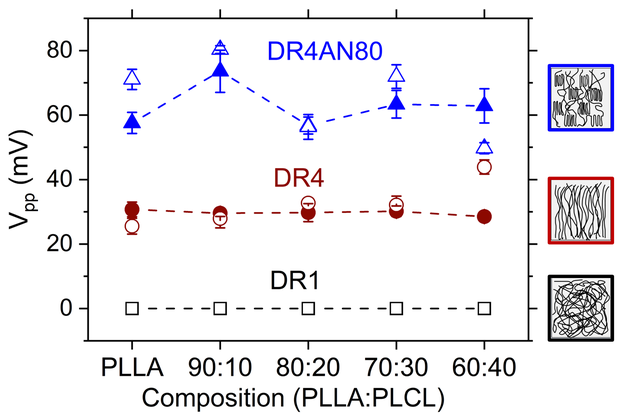
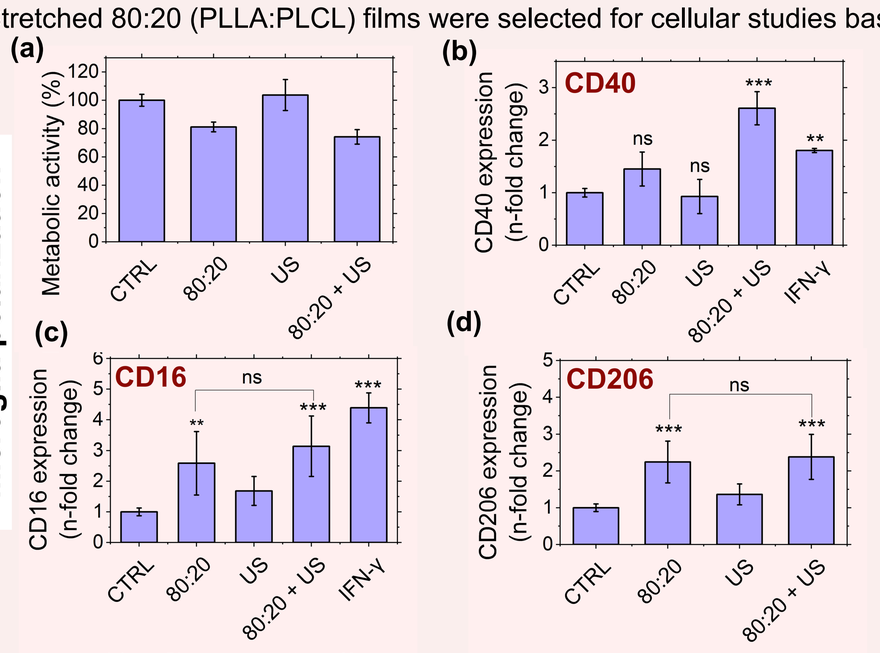


Figure 6. Piezoelectric response evaluated by measuring voltage output in the d_{14} shear-mode. **Orientation and crystallinity are directly correlated with piezoelectric response.**

Microglia polarization

Stretched 80:20 (PLLA:PLCL) films were selected for cellular studies based on their optimal balance of functional properties and piezoelectric activity.



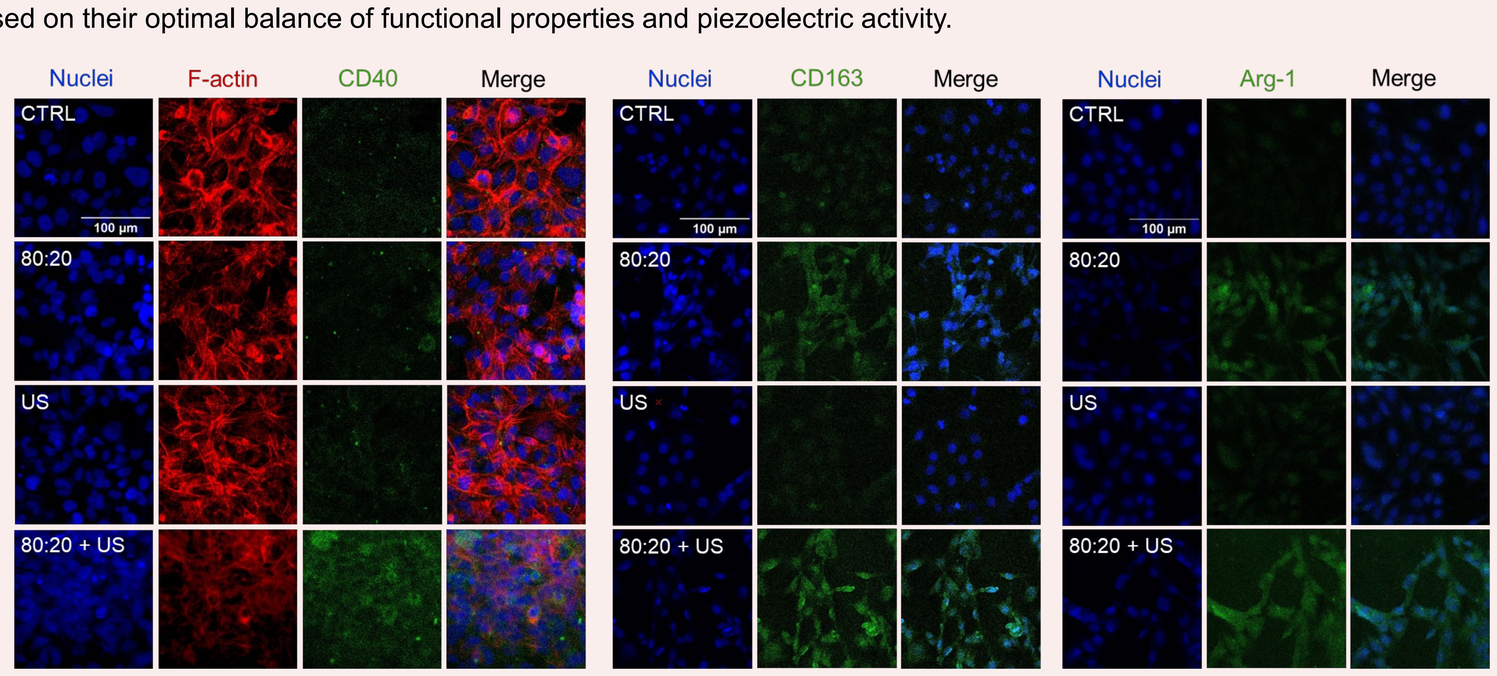


Figure 7. (a) Metabolic activity evaluated by WST-1. Flow-cytometry results are shown as fold change with respect to control (non-stimulated) cells (ns $p > 0.5$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Figures represent (b) CD40 (c) CD16 (d) CD206 surface markers.

Figure 8. Immunofluorescence imaging with (a) CD40 and (b) CD163 surface, and (c) Arg-1 intracellular markers.

CONCLUSION

PLCL can be incorporated up to 40 wt.% without compromising the piezoelectric response. Enables:

- ✓ Modulation of mechanical properties → reduced stiffness and increased ductility and toughness.
- ✓ Enhanced thermal stability → increased processability via advanced manufacturing techniques (e.g., melt electrowriting or 3D printing).

US-activated PLLA:PLCL piezoelectric blends (80:20 composition) can modulate microglial polarization:

- ✓ No cytotoxicity was observed, although cells on the substrate showed reduced metabolic activity, likely reflecting lower initial adhesion.
- ✓ CD40 expression increased under US stimulation on the 80:20 piezoelectric films, consistent with a piezoelectric contribution.
- ✓ The substrate alone was associated with increased CD206, CD163, and Arg-1 expression, suggesting a substrate-driven anti-inflammatory shift.

Overall, these findings support bioresorbable PLLA-based piezoelectric films as promising immunomodulatory platforms for dynamic control of microglial phenotype. This may be valuable in applications such as immunotherapy in brain tumors, where a transient pro-inflammatory stimulus is beneficial, but excessive neuroinflammation must be controlled [2].

REFERENCES

[1] Battaglini M et al. *Adv Healthc Mater.* 2024;13(12):e2304180.

[2] Schönlein R et al. *Biomaterials.* 2026;324:123522.

ACKNOWLEDGMENTS

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