Ascorbic acid-based eutectogels as immunomodulatory platforms: microglial polarization modulation and precision fabrication via volumetric 3D printing

Sara Martín-Colomo[1,2], Matías L. Picchio[1,3], Quinten Thijssen[4], Sandra Van Vlierberghe[4], Jose Ramon Pineda[2], Aitor Larrañaga[1]

[1]Department of Mining-Metallurgy Engineering and Materials Science, POLYMAT, Bilbao School of Engineering, University of the Basque Country (UPV/EHU), 48013, Bilbao, Spain. [2]Department of Cell Biology and Histology, University of the Basque Country UPV/EHU, 48940, Leioa, Spain. [3]Ikerbasque, Basque Foundation for Science, 48009, Bilbao,Spain. [4]Polymer Chemistry & Biomaterials Group, Centre of Macromolecular Chemistry, Department of Organic and Macromolecular Chemistry, Ghent University, 9000, Ghent, Belgium

sara.martinc@ehu.eus

ABSTRACT

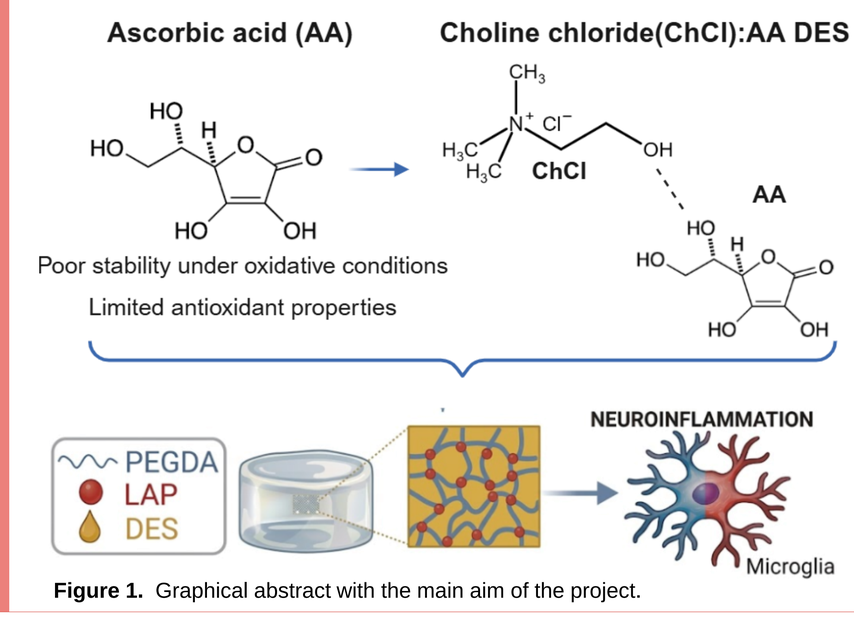


Figure 1. Graphical abstract with the main aim of the project.

METHODS

Table 1. Eutectogels formulations

	PEGDA (% v/v)
10PEGDA:DES	10
30PEGDA:DES	30
50PEGDA:DES	50

Results were analyzed as mean ± standard deviation (SD).

One-way analysis of variance (ANOVA) was applied as statistical analysis using a confidence interval of 95% ($p<0.05$).

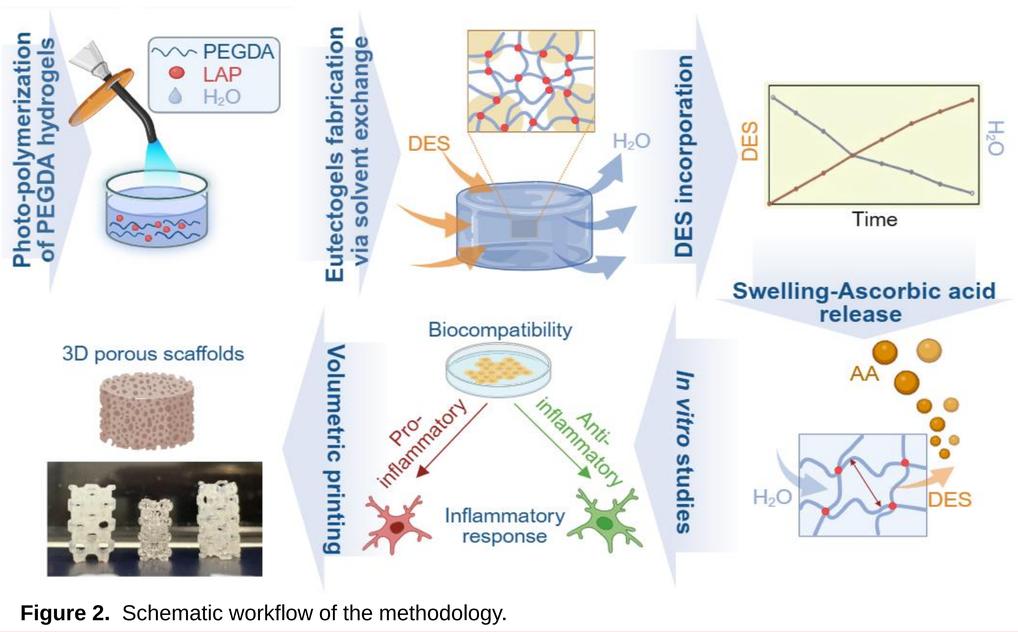


Figure 2. Schematic workflow of the methodology.

DES INCORPORATION

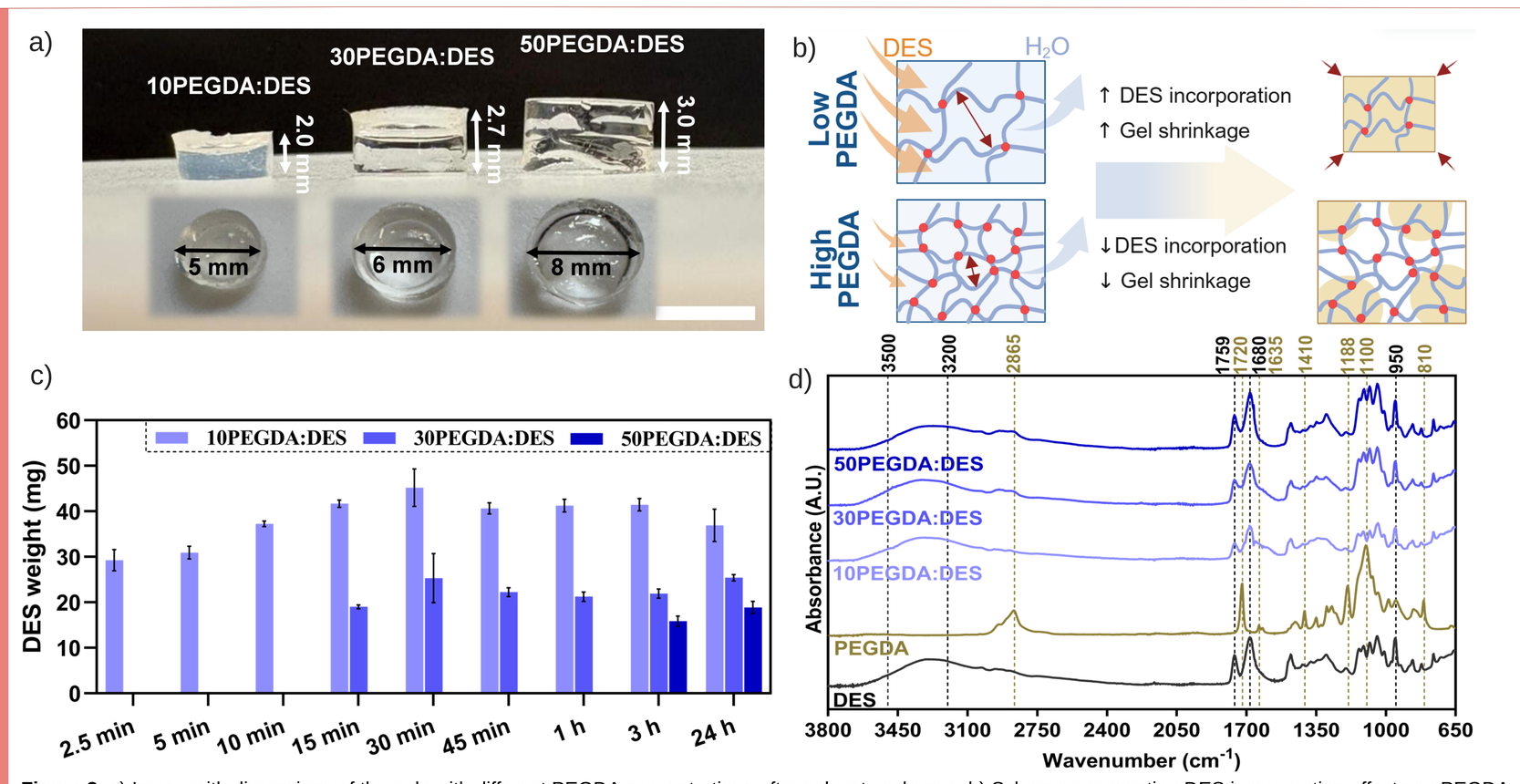


Figure 3. a) Image with dimensions of the gels with different PEGDA concentrations after solvent exchange. b) Scheme representing DES incorporation effects on PEGDA matrices. c) Gravimetric analysis showing DES incorporation over 24 h. d) FTIR of the different PEGDA:DES eutectogels, raw PEGDA solution and ChCl:AA DES.

SWELLING-AA RELEASE

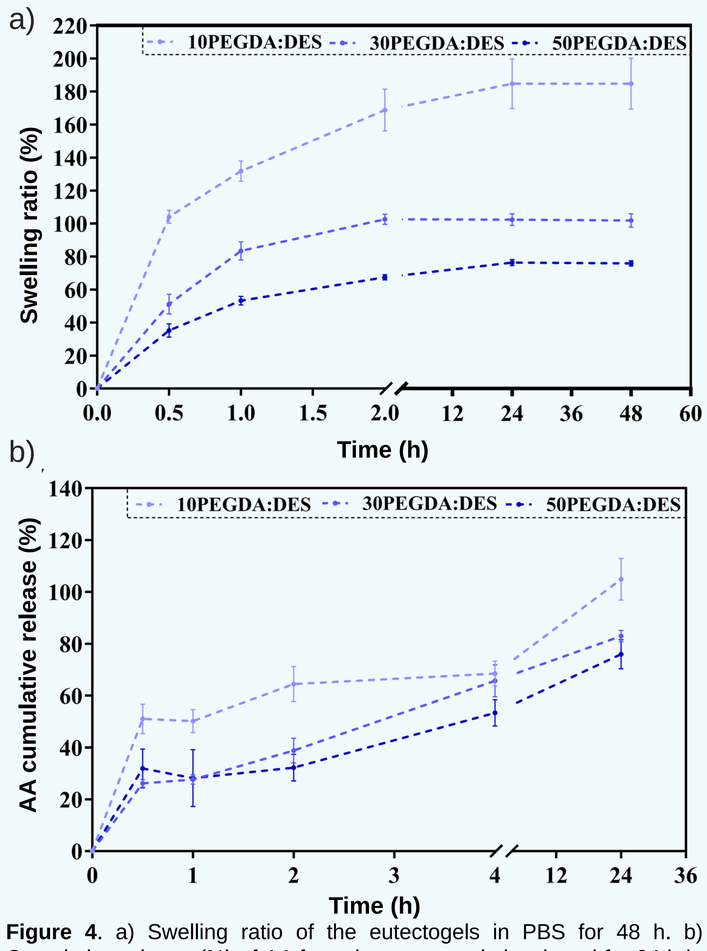


Figure 4. a) Swelling ratio of the eutectogels in PBS for 48 h. b) Cumulative release (%) of AA from the eutectogels incubated for 24 h in PBS.

IN VITRO STUDIES

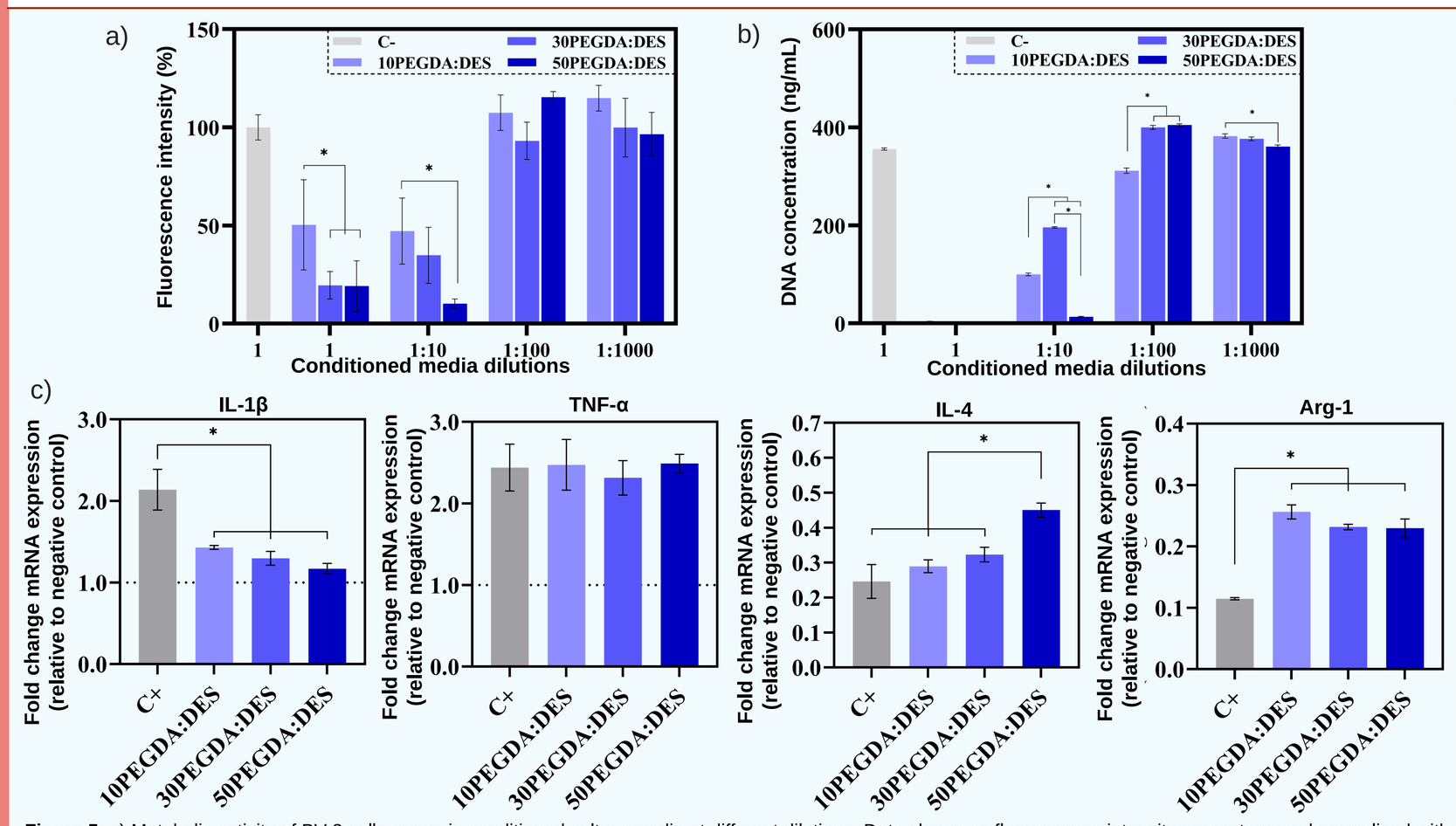


Figure 5. a) Metabolic activity of BV-2 cells grown in conditioned culture media at different dilutions. Data shown as fluorescence intensity percentage and normalized with respect to the control (C-). b) DNA concentration of BV-2 cells grown in conditioned culture media at different dilutions. c) mRNA expression of pro-inflammatory genes TNF-α, IL-1β and anti-inflammatory genes IL-4, Arg-1. mRNA expression is presented as $2^{-\Delta\Delta Ct}$ value and normalized with respect to the SA control (fold change = 1).

VOLUMETRIC PRITNING

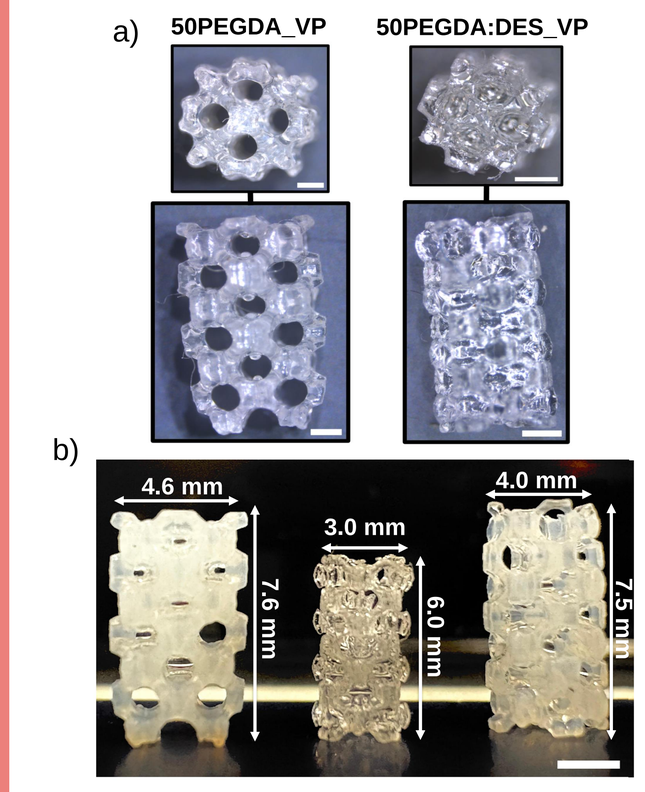


Figure 6. a) 50PEGDA_VP and 50PEGDA:DES_VP photos. Scale bars of 1 mm. d) Image of 50PEGDA_VP, 50PEGDA:DES_VP and 50PEGDA:DES_VP after 24 h in PBS 1x (from left to right). Scale bar of 2 mm. VP refers to volumetric printing.

CONCLUSIONS

- ChCl:AA DES was incorporated into PEGDA matrices, yielding antioxidant eutectogels with immunomodulatory potential.
- The polymeric network played a key role in the uptake of DES, with lower network density enhancing incorporation and inducing gel shrinkage.
- The high swelling capacity of the eutectogels guided a controlled release of AA.
- Released AA modulated microglial response under LPS stimulation, promoting an anti-inflammatory phenotype with upregulated IL-4 and Arg-1 genes.
- Volumetric printing allowed the fabrication of 3D PEGDA scaffolds that supported DES incorporation while preserving structural integrity.
- Overall, DES-based eutectogels represent a promising platform as immunomodulatory biomaterials, enabling the preservation of the antioxidant activity of AA with a controlled release, and an effective modulation of microglial response in neuroinflammatory environments.

REFERENCES

[1] Silva, Joana M., et al. ACS Sustain. Chem. Eng. 6.8 (2018): 10355-10363.
[2] Mercadal, Pablo A., et al. *JACS Au* 4.10 (2024): 3744-3758.
[3] Loane, David J., and Alok Kumar. Exp. Neurol. 275 (2016): 316-327.
[4] Xu, Liju, et al. Adv. Mater. 32.52 (2020): 2004579.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the financial support from the University of the Basque Country (UPV/EHU) collaborative project COLAB24/EHU-G24/08 and for the predoctoral grant of Sara Martin-Colomo (PIF22/119). Authors are also thankful for funds from the Basque Government, Department of Education (IT-1766-22 and IT-1751-22).