Self-assembled three-dimensional hydrogels based on graphene derivatives and cerium oxide nanoparticles: scaffolds for long-term co-culture of oligodendrocytes and neurons derived from neural stem cells

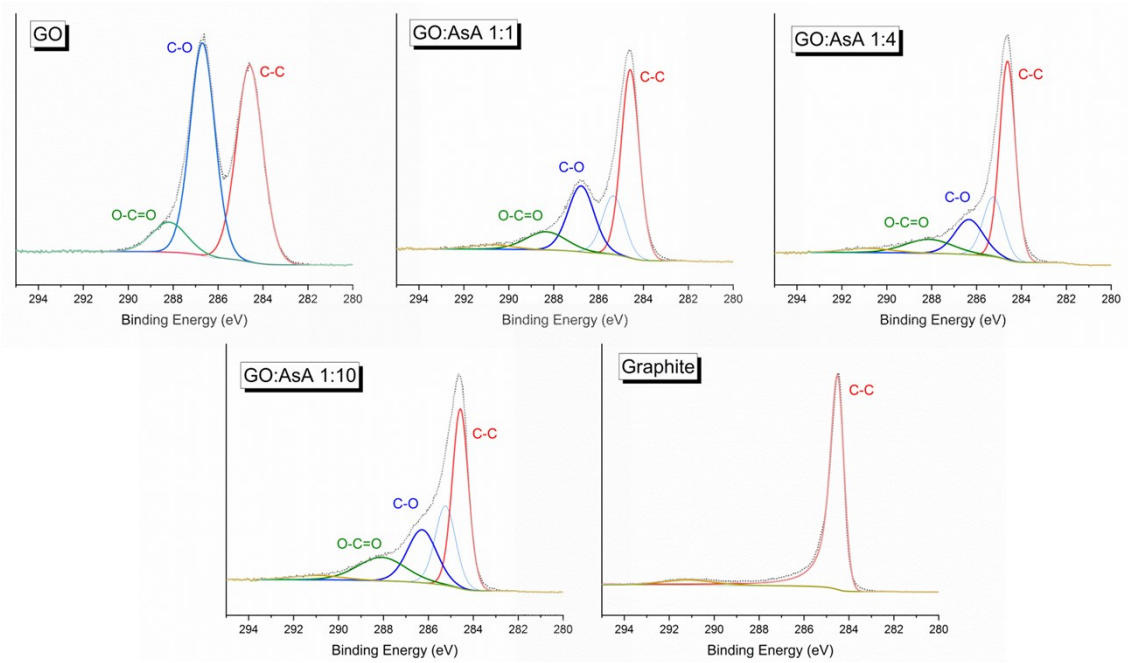
Yurena Polo[a], Jon Luzuriaga[b], Sergio Gonzalez de Langarica[c], Beatriz Pardo-Rodríguez[b], Daniel E. Martínez-Tong[d], Christos Tapeinos[e,f], Irene Manero-Roig[b,g], Edurne Marin[c], Jone Muñoz-Ugartemendia[c], Gianni Ciofani[e], Gaskon Ibarretxe[b], Fernando Unda[b], Jose-Ramon Sarasua[c], Jose Ramon Pineda[*b, h], Aitor Larrañaga[*c]

[a] *Polimerbio, Donostia-San Sebastian, Spain*
[b] *Cell Signaling lab, Department of Cell Biology and Histology, Faculty of Medicine and Nursing, University of the Basque Country (UPV/EHU), Leioa, Spain*
[c] *Group of Science and Engineering of Polymeric Biomaterials (ZIBIO Group), Department of Mining, Metallurgy Engineering and Materials Science, POLYMAT, University of the Basque Country (UPV/EHU), Bilbao, Spain*
[d] *Polymers and advanced materials: Physics, Chemistry and Technology, University of the Basque Country (UPV/EHU), Donostia-San Sebastian, Spain & Centro de Física de Materiales (UPV/EHU-CSIC), Donostia-San Sebastian, Spain*
[e] *Smart Bio-Interfaces, Istituto Italiano di Tecnologia, Pontedera (PI), Italy*
[f] *Division of Pharmacy and Optometry, School of Health Sciences, Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK*
[g] *Université de Bordeaux IINS - UMR 5297, Bordeaux, France*
[h] *Achucarro Basque Center for Neuroscience Fundazioa, Leioa, Spain*

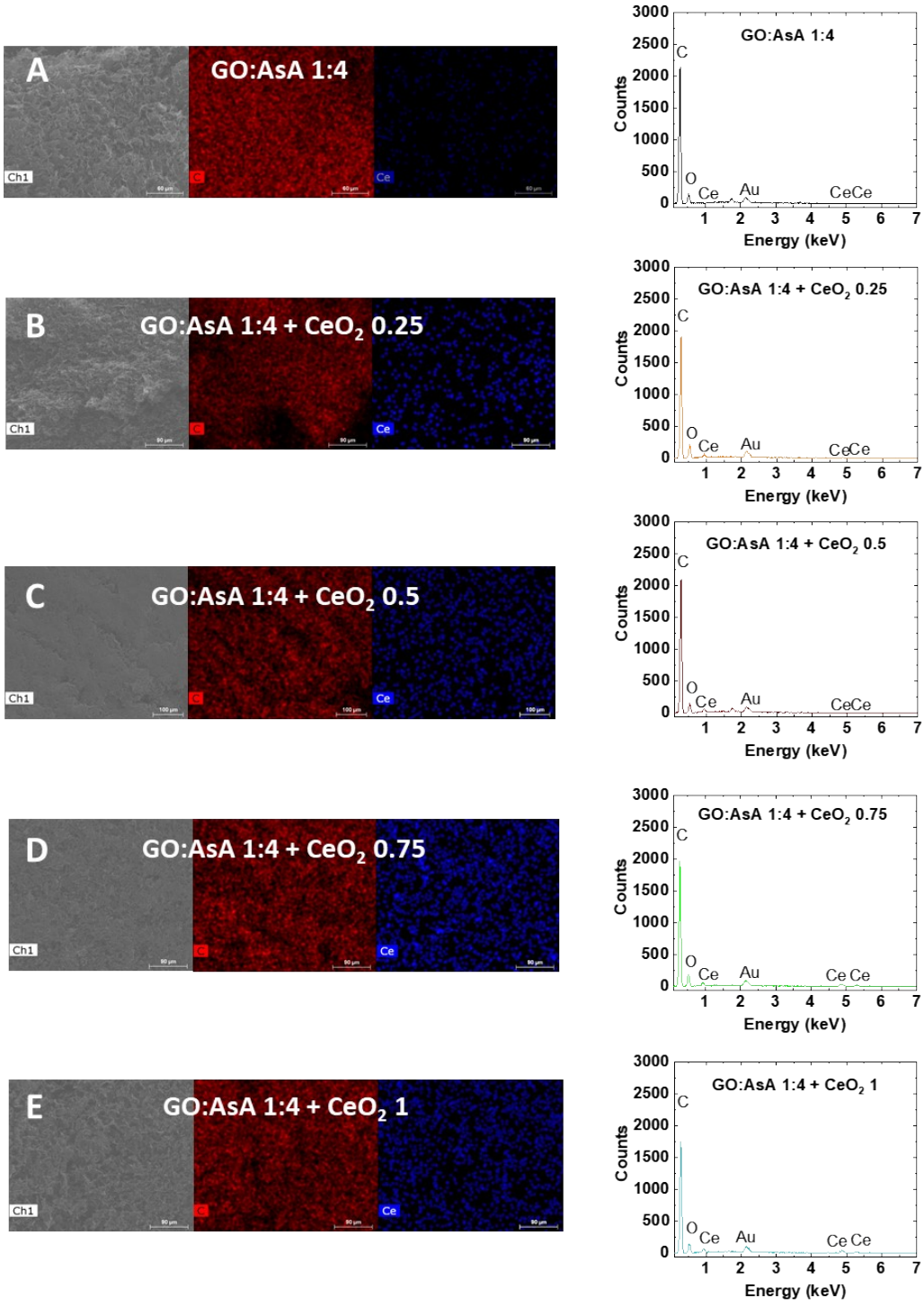
[*] ***Corresponding Authors:***
Aitor Larrañaga
Group of Science and Engineering of Polymeric Biomaterials (ZIBIO Group), Department of Mining, Metallurgy Engineering and Materials Science & POLYMAT, University of the Basque Country (UPV/EHU), 48013 Bilbao, Spain
Telephone: +34 946 013 935
E-mail: aitor.larranagae@ehu.eus
ORCID: 0000-0002-2123-6069

Jose Ramon Pineda
Cell Signaling lab, Department of Cell Biology and Histology, Faculty of Medicine and Nursing, University of the Basque Country (UPV/EHU), 48940 Leioa, Spain
Telephone: +34 946 012 426
E-mail: joseramon.pinedam@ehu.eus
ORCID: 0000-0002-0900-7466

Supplementary figures

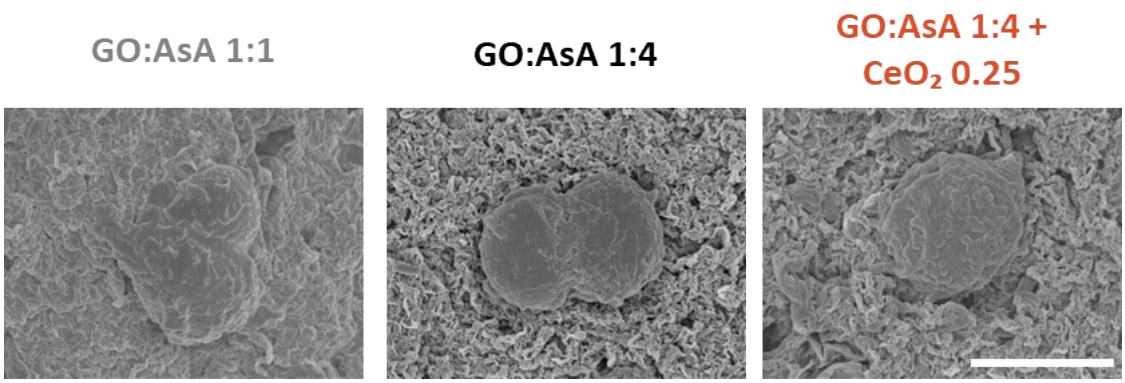


Supplementary figure 1: XPS spectra of the samples.
High resolution C 1s spectra for GO, GO:AsA 1:1, GO:AsA 1:4, GO:AsA 1:10 and graphite, highlighting the different functionalities that were found, based on the deconvolution of the signal.



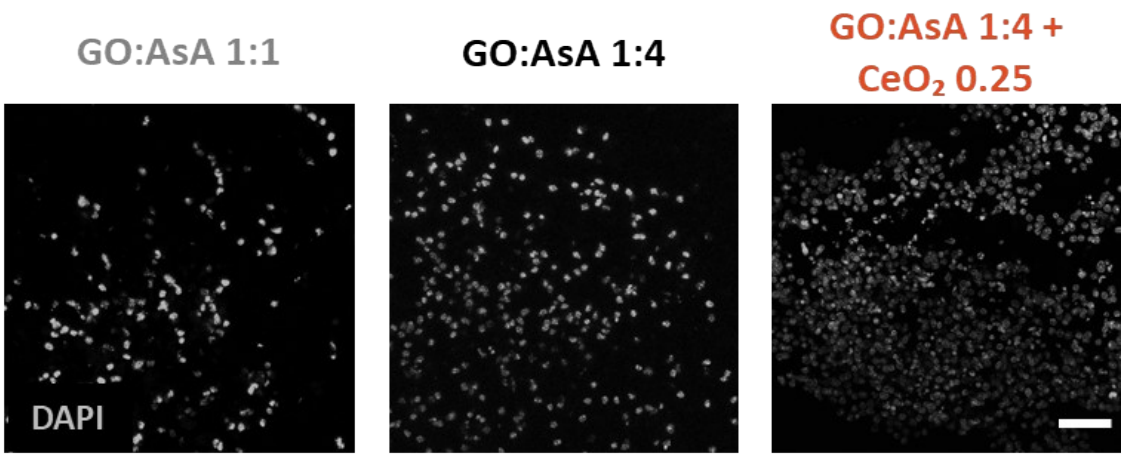
Supplementary figure 2: EDX mapping and spectra of the hydrogels containing increasing amounts of CeO_2 nanoparticles.

(A) GO:AsA 1:4 (B) GO:AsA 1:4 + CeO_2 0.25 (C) GO:AsA 1:4 + CeO_2 0.5 (D) GO:AsA 1:4 + CeO_2 0.75 and (E) GO:AsA 1:4 + CeO_2 1 showing the peaks of the C, O and Ce.



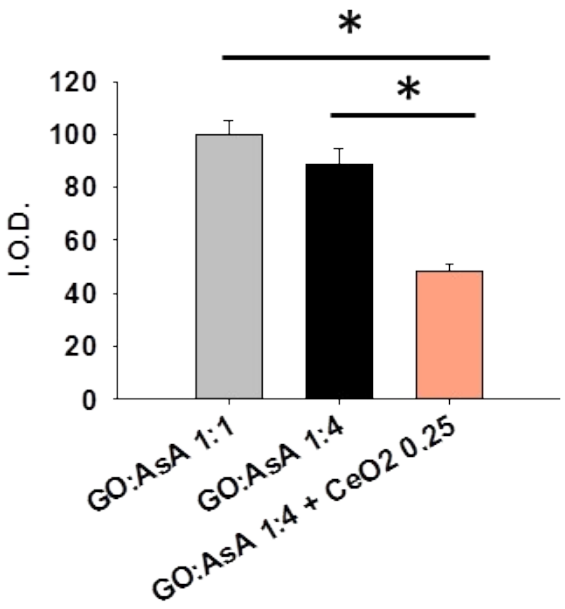
Supplementary figure 3: Scanning electron micrographs showing attachment of the cells over the 3D structure.

All the hydrogels GO:AsA 1:1, GO:AsA 1:4 and GO:AsA 1:4 + CeO_2 0.25 supported NSC attachment after 24 h. Scale bar 10 µm.

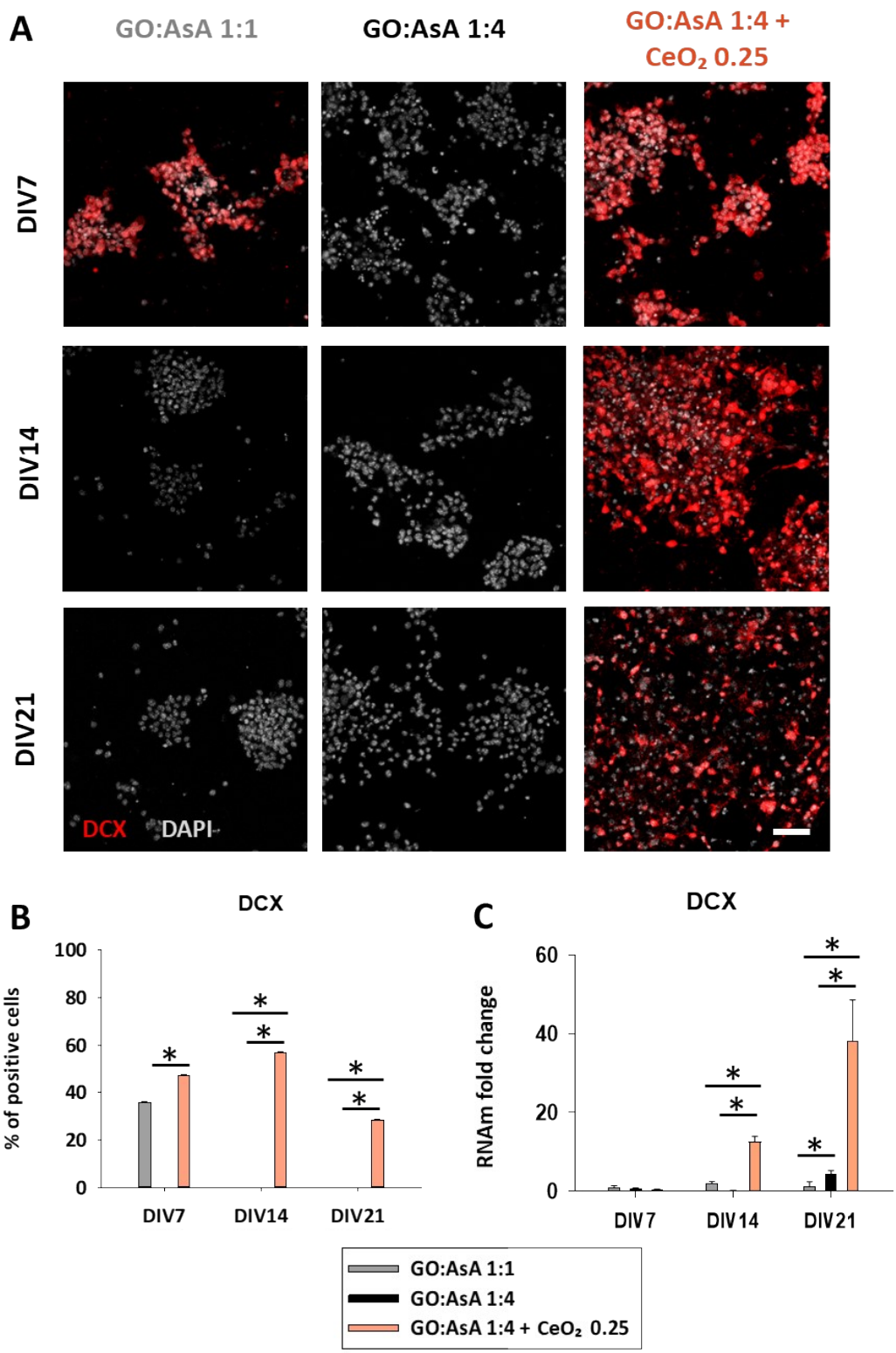


Supplementary figure 4: **Immunofluorescence images showing the integration of the cells over the 3D structure.**

Immunofluorescence images for DAPI on hydrogel pieces cut by the vibratome showed cells until 500 µm deep after 7 days in culture on the GO:AsA 1:1, GO:AsA 1:4 and GO:AsA 1:4 + CeO_2 0.25. Scale bar 50 µm. Briefly, hydrogels were embedded in 2% agarose and serial cut in 250 µm thickness sections using a vibratome. By using serial cut hydrogel sections, we are able to do a hydrogel reconstruction from top to bottom with a precise micrometric measure of depth of DAPI immunofluorescent signal detection.



Supplementary figure 5: Intracellular reactive oxygen species measurements.
Quantification of the intracellular reactive oxygen species on NSCs cultured for 14 days over GO:AsA 1:1, GO:AsA 1:4 and GO:AsA 1:4 + CeO_2 0.25 hydrogels. (*$p<0.05$ compared to GO:AsA 1:1 and GO:AsA 1:4, Holm-Šídák method One Way Analysis of Variance on Rank).



Supplementary figure 6: **Immunofluorescence showing immature neuronal cells on the hydrogels at different time points**.

(A) Immunofluorescence showing immature DCX (red) positive neuronal-like cells in GO:AsA 1:1, GO:AsA 1:4 and GO:AsA 1:4 + CeO_2 0.25 hydrogels after DIV 7, 14 and 21. Scale bar 50 µm. (B) Quantification of the percentage of DCX positive cells (C) qPCR measurements showing the fold change compared to GO:AsA 1:1 hydrogel at DIV7. (*$p<0.05$ compared to the other hydrogels at the same time-points. Holm-Šídák method One-way ANOVA Analysis of Variance on Ranks).