# 3D free-standing porous scaffolds made of graphene oxide as substrates for neural cell growth

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## Electronic Supplementary Information

### Further discussion on GOx characterization

Thermal treatment of GOx promoted a severe loss of mass (*ca.* 40 %, as deduced from TGA) corresponding to the decomposition of oxygen functional groups, as both FT-IR and XPS revealed. The initial oxygen content of the GOx synthesized was very high (C/O ratio of 2.1, as determined by XPS). This oxygen was mostly in the form of hydroxyl and epoxy (63 %) and carbonyl or carboxyl (37 %) groups, according to XPS and confirmed by FT-IR. However, after thermal treatment, the oxygen content significantly decreased (C/O ratio of 4.3). At the same time, the intensities of the FT-IR peaks corresponding to the oxygen functionalities (*e.g.*, vibration and deformation peaks of O–H groups at 3395 $cm^{-1}$ and 1410 $cm^{-1}$, respectively, and the C–O (alkoxy) stretching peak at 1052 $cm^{-1}$) decreased dramatically, suggesting that most O-H functional groups were removed.[1] Besides, the peak at 1578 $cm^{-1}$ (corresponding to the skeletal vibration of graphene)[2] appeared, being then noticeable the restoration of the

$Csp^2$ structure in this thermally treated GOx even after only 200 °C.[3] The increase in the intensity of the band at 1226 $cm^{-1}$ may be ascribed to either the epoxide ring opening followed by the inter/intramolecular lactone/ester formation[4] or the combination of free hydroxyl groups and K-O stretching/hydroxyl groups, supporting the reaction between potassium ions and oxygen functional groups of GOx sheets.[5]

**Table 1-SI. Characterization of GOx samples by Raman spectroscopy.**

| Powder | Graphite | GOx | Cross-linked GOx | Cross-linked & thermally treated GOx | Thermally treated GOx |
|---|---|---|---|---|---|
| $I_D/I_G$[a] | 0.09 | 0.77 | 0.79 | 0.81 | 0.78 |
| $W_D(cm^{-1})$[b] | 1361 | 1355 | 1354 | 1354 | 1358 |
| $W_G(cm^{-1})$[c] | 1581 | 1590 | 1587 | 1596 | 1595 |

[a] Ratio between the intensity of bands D and G in the Raman spectra.

[b] Wavelength at which band D shows the maximum.

[c] Wavelength at which band G shows the maximum.

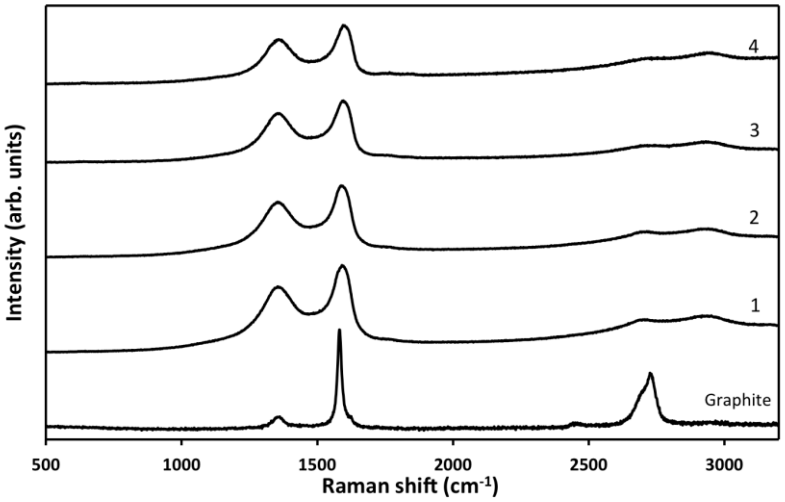


**Figure 1-SI.** Representative Raman spectra of GOx as obtained (1), HMDI-cross-linked GOx (2), HMDI-cross-linked GOx thermally reduced at 200 ºC (3) and GOx thermally reduced at 200 ºC (4). Raman spectrum of pristine Graphite is also included for comparison.

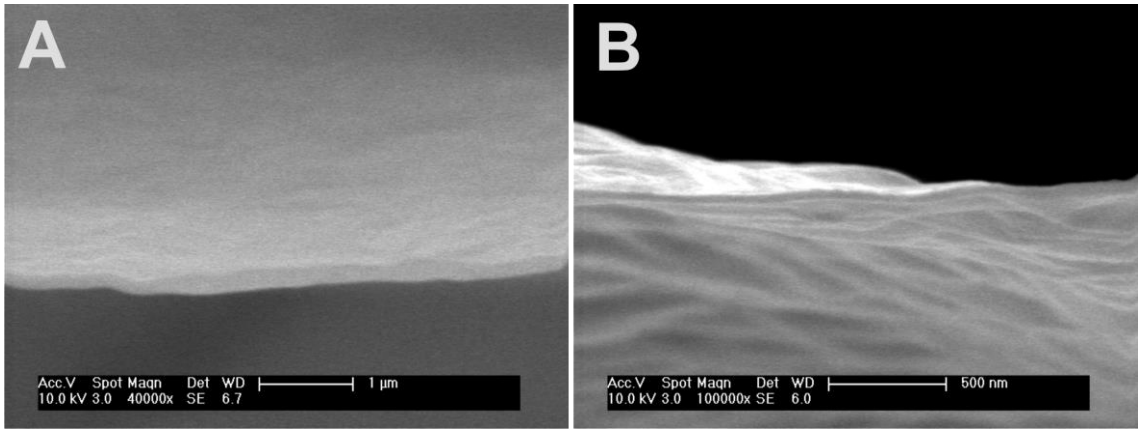


**Figure 2-SI.** Field-emission SEM images illustrating a detail of the wrinkled sheets that conforms the walls of pristine (A) and cross-linked and thermally treated 3D GOx scaffolds (B).

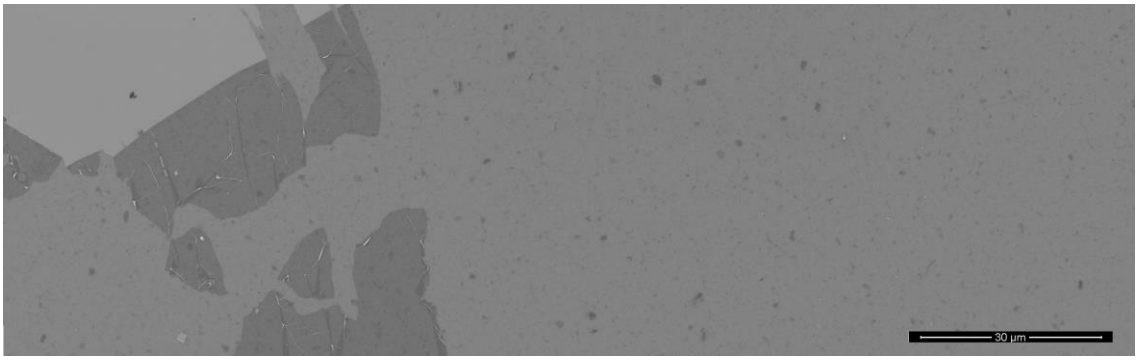


**Figure 3-SI.** Panoramic view of a homogeneous 2D GOx film after thermal treatment at 200 ºC as obtained by field-emission SEM.

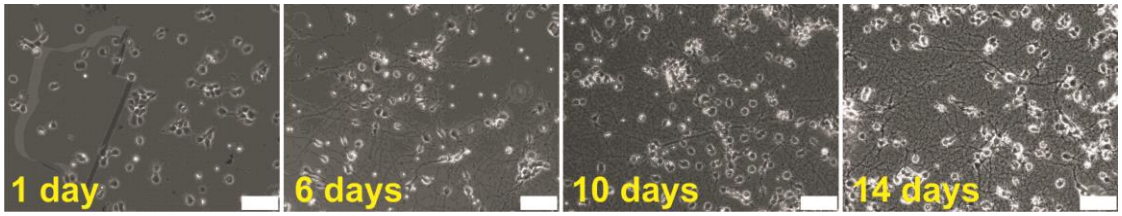


**Figure 4-SI.** Progressive formation of a neural network by ENPCs on *2D films*. Pictures correspond to representative optical microscopy images at different time points. Scale bars represent 25 µm in all cases.

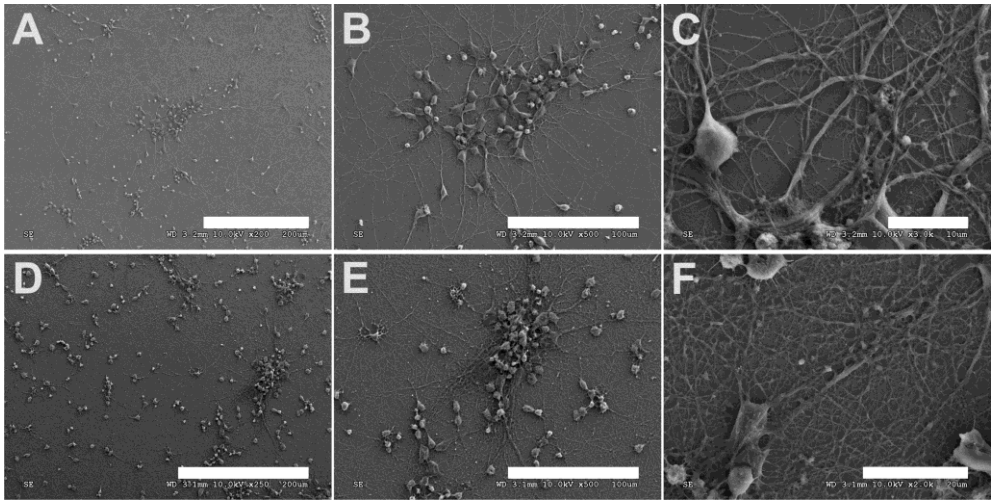


**Figure 5-SI.** SEM images of representative ENPC cultures on PL-coated glass coverslips (*control*) at either 7 (A-C) or 14 (D-F) days. Scale bars represent 200 μm (A,D), 100 μm (B,E), 20 μm (F), and 10 μm (C).

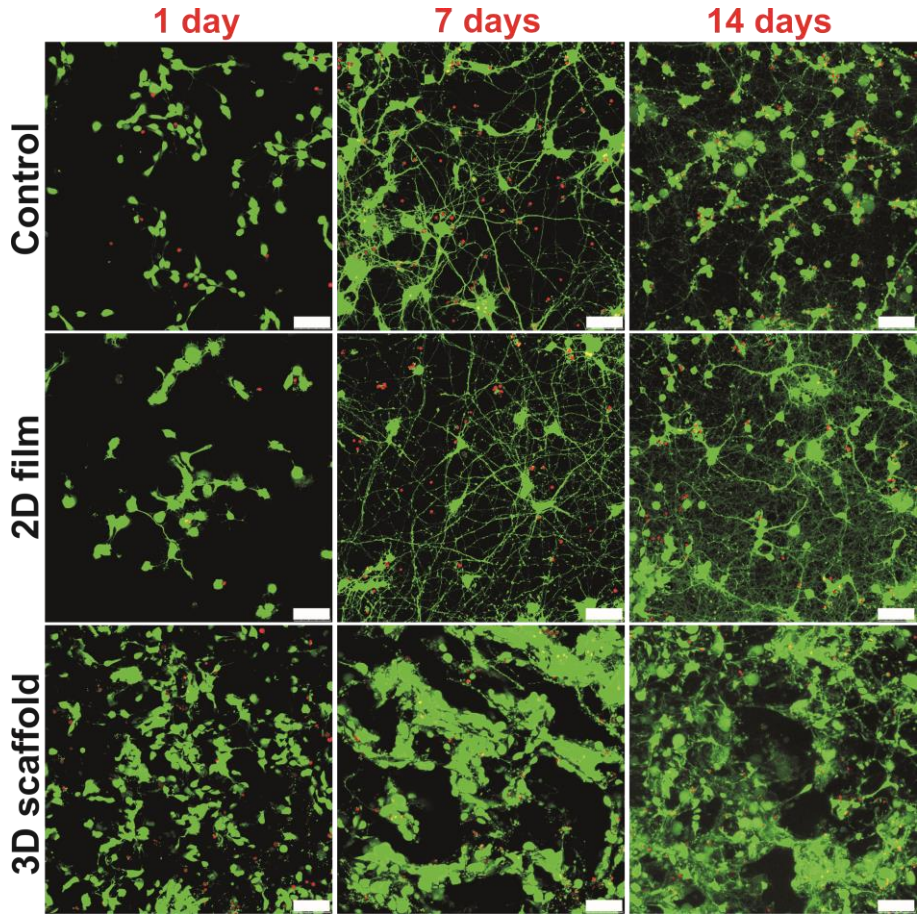


**Figure 6-SI.** ENPC viability studies on GOx-based substrates over time. Representative CLSM images are shown. Alive cells appear in green (stained with calcein), while dead cells are shown in red (EthD-1). Scale bars represent 50 μm in all images.

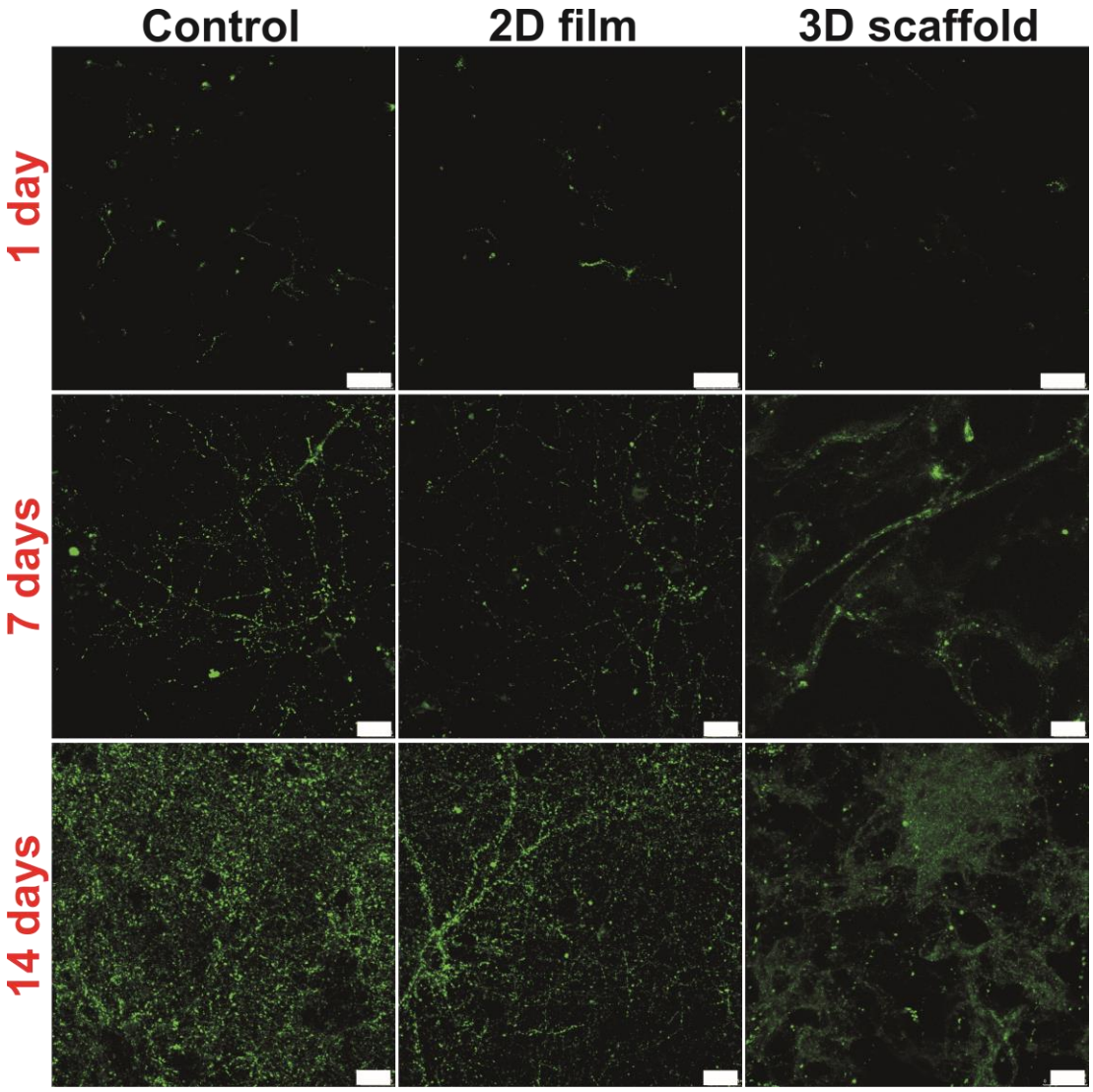


**Figure 7-SI.** Synapsis formation on GOx-based substrates. Representative CLSM images showing staining for synaptophysin (green) at different time points are shown. Scale bars represent 50 μm in all images.

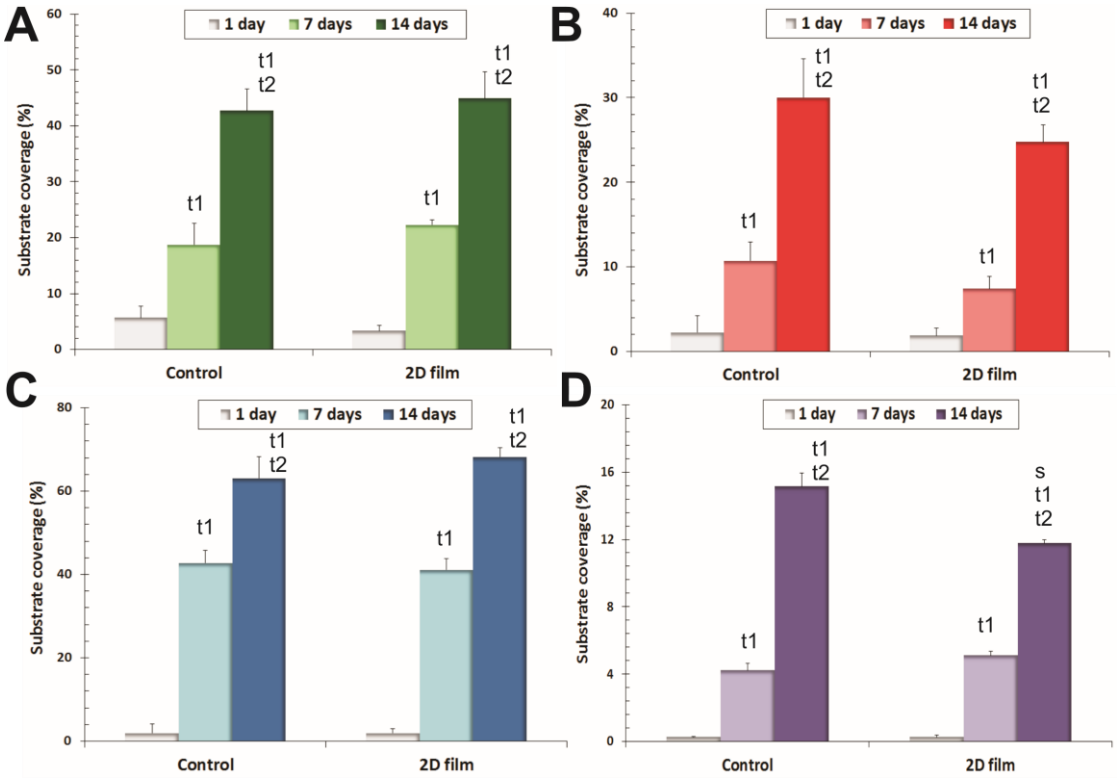


**Figure 8-SI.** Quantification of ENPC differentiation on *control* and *2D films* over time. Histograms show the percentage of substrate area positively stained for the different markers: map-2 (A), vimentin (B), tau (C), and synaptophysin (D). Statistically significant differences of each sample with respect to 1 day (*t1*), 7 days (*t2*) and *control* (*s*) are indicated ($p < 0.05$).

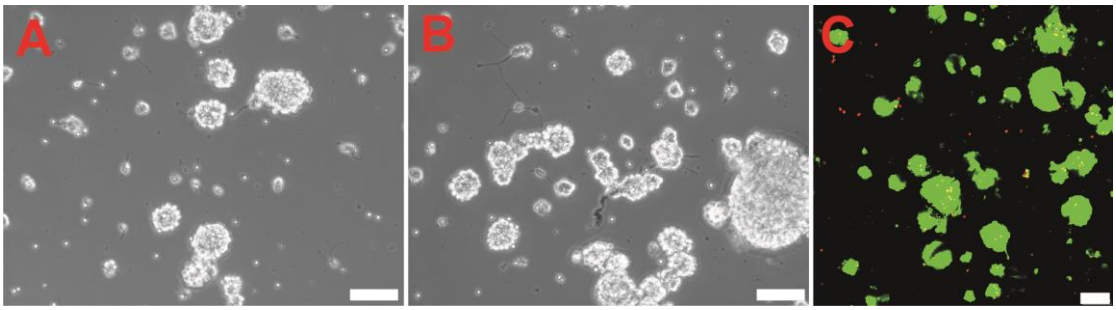


**Figure 9-SI.** Demonstration of the need of PL-coating to guarantee appropriate ENPC adhesion and growth on GOx substrates. Representative images of ENPC cultures at 2 days on a glass coverslip (A), 2D GOx-based film (B) and 3D GOx-based scaffold (C) without coating of PL are shown. Pictures are obtained by either optical microscopy (A,B) or CLSM after viability staining (C). Scale bars represent 25 μm (A,B) and 50 μm (C).

## References


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