Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information

Interaction of graphene and WS_2 with neutrophils and mesenchymal stem cells: implications for peripheral nerve regeneration

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Raman analysis of as-grown samples

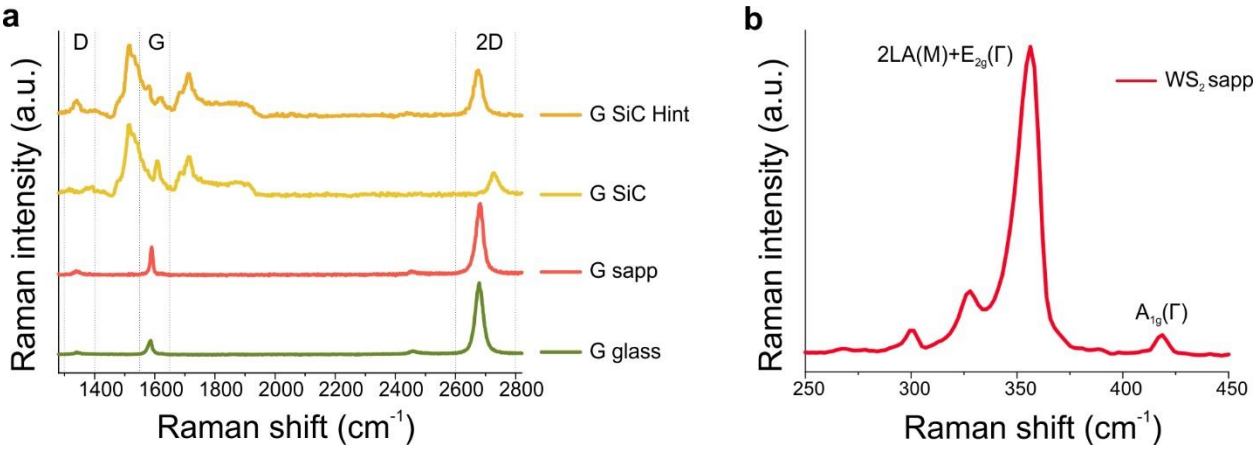

Figure S1. Characteristic Raman spectra of the as-grown graphene samples (a) and CVD WS_2 (b). (a) Raman spectra of CVD graphene on copper transferred on glass (G glass), graphene grown on sapphire (G sapp), epitaxial graphene on SiC (G SiC) and hydrogen-intercalated graphene on SiC (G SiC Hint). The ranges of the D, G and 2D bands are indicated with dashed lines. The G peak was found at ~1580-1600 cm^{-1} and the 2D peak at ~ 2680-2720 cm^{-1}, both position ranges were comparable to those reported for similar samples in previous works [1–4]. Graphene on SiC showed additional peaks in the 1000 cm^{-1} to 2000 cm^{-1} range, due to the SiC substrate Raman signal. (b) Representative Raman spectrum of CVD WS_2 on sapphire (WS_2 sapp). The 2LA(M) + E_{2g}(Γ) peaks at ~ 355 cm^{-1} and the A_{1g}(Γ) peak at ~ 418 cm^{-1} are highlighted.

AFM analysis after incubation with cell medium

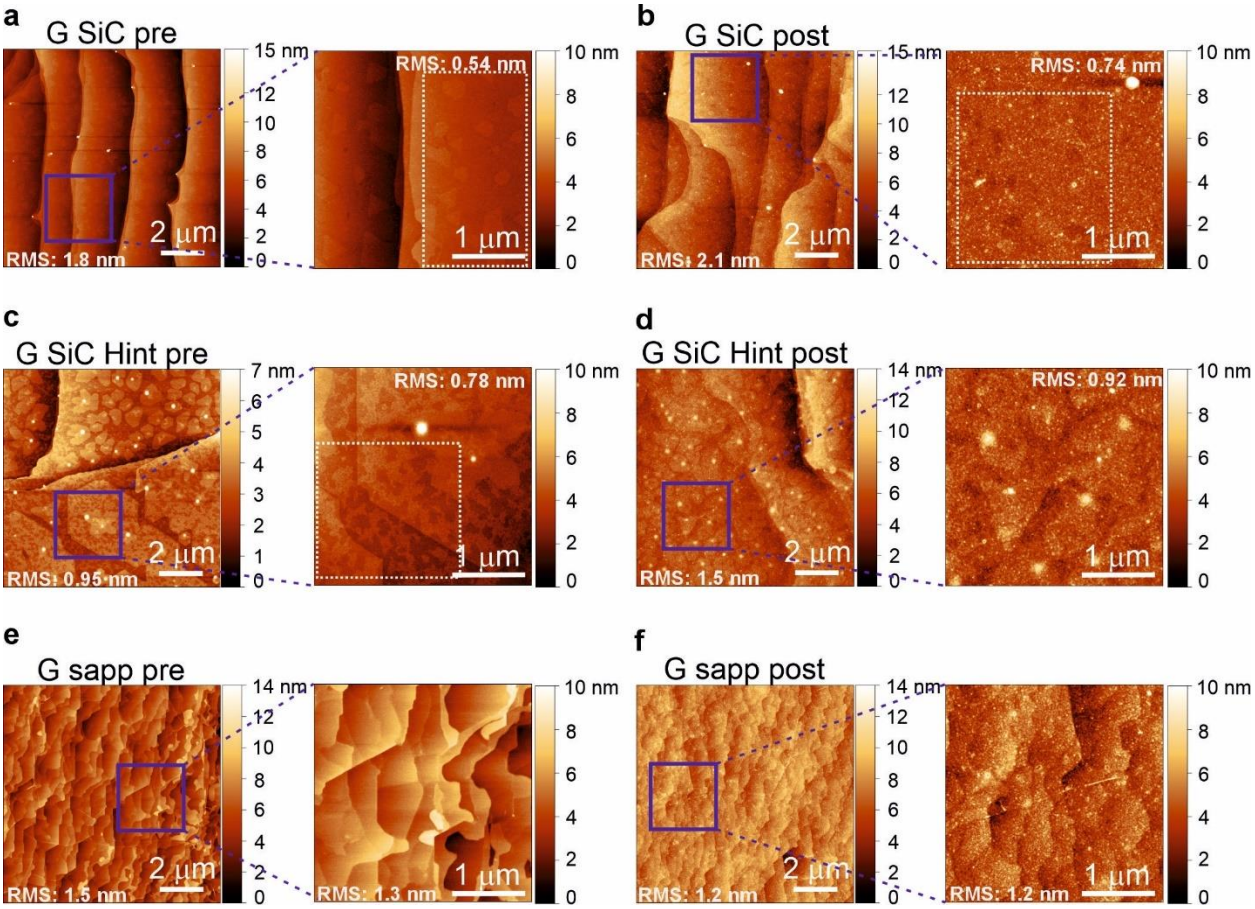

Figure S2. AFM topography of epitaxial graphene on SiC (a,b, G SiC), hydrogen-intercalated graphene on SiC (c,d, G SiC Hint) and graphene grown on sapphire (e,f, G sapp) before (pre) and after 24 h incubation with cell culture medium (post). After the incubation all the samples are coated with a carpet of nanometric spots. The estimated RMS roughness for the three samples before the incubation is approximately 1-2 nm, mainly due to the presence of the nanometric terraces which are formed after the hydrogen etching. For all the graphene samples the RMS increased after the incubation, due to the presence of the nanometric spots.

Additional NETosis analysis

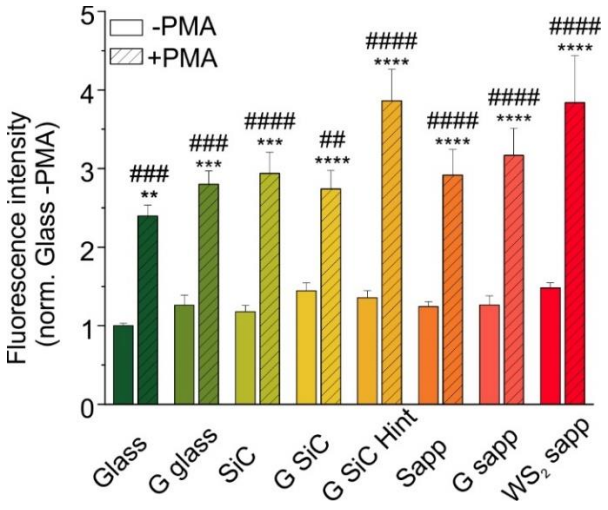

Figure S3. NETosis of neutrophil-like differentiated HL60 with and without PMA treatment after 4h incubation with graphene and WS_2. Fluorescent intensity of NETs visualized as extracellular DNA (Syto green). The intensities are reported in comparison to an assigned value of 1 for the DNA released by neutrophils on the glass control without PMA. The fluorescence intensity more than doubled after PMA treatment for all the substrates. Data reported as mean + standard error of the mean (s.e.m.). One-way ANOVA with Dunnet post hoc test was used for statistical significance to compare each substrate with the glass control, with **$p < 0.01$, ***$p < 0.001$****$p < 0.0001$. One-way ANOVA with Sidak post hoc test was used for statistical significance to compare each PMA treated substrate with the non-treated one, with ##$p < 0.01$, ###$p < 0.001$, ####$p < 0.0001$.

Contact angle measurements

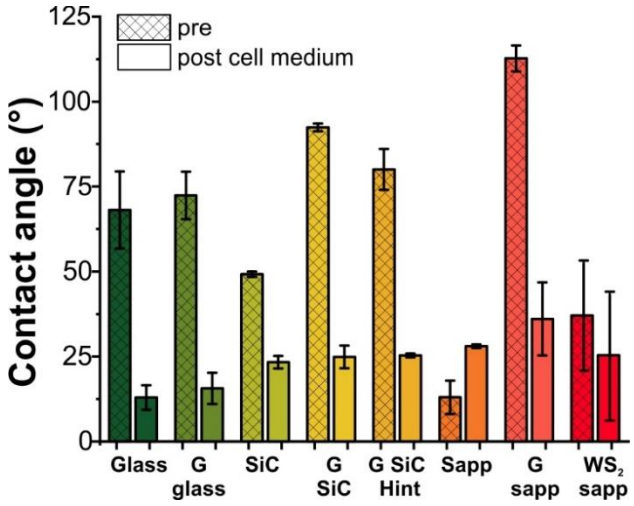

Figure S4. Contact angle measurements of graphene and WS_2 before and after one day incubation with cell medium. Values are the mean ± standard deviation (SD) for 2 samples. As-grown graphene samples are more hydrophobic than as-grown WS_2 and the control substrates. However, following 24 h incubation with cell medium, all the samples showed an increased hydrophilicity probably due to the presence of medium residuals on the surface.

Contact angles were measured using a CAM 101 contact angle meter, from KSV Instruments Ltd. (Finland) and estimated by measuring the angles between the baseline of the droplet and the tangent at the droplet boundary, using DI water as a probe liquid.

Additional Raman analysis after neutrophil incubation

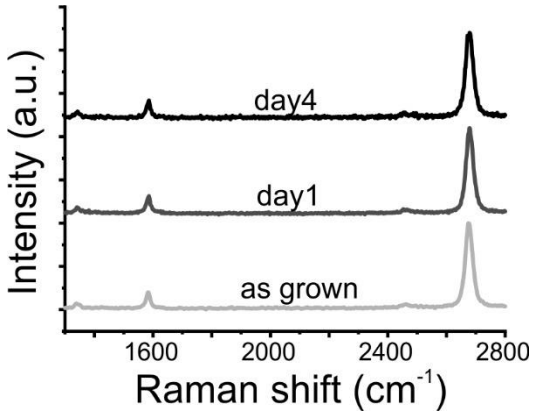

Figure S5. Characteristic Raman spectra of graphene grown directly on glass before and after treating with neutrophil-like differentiated HL60 for 1 and 4 days.

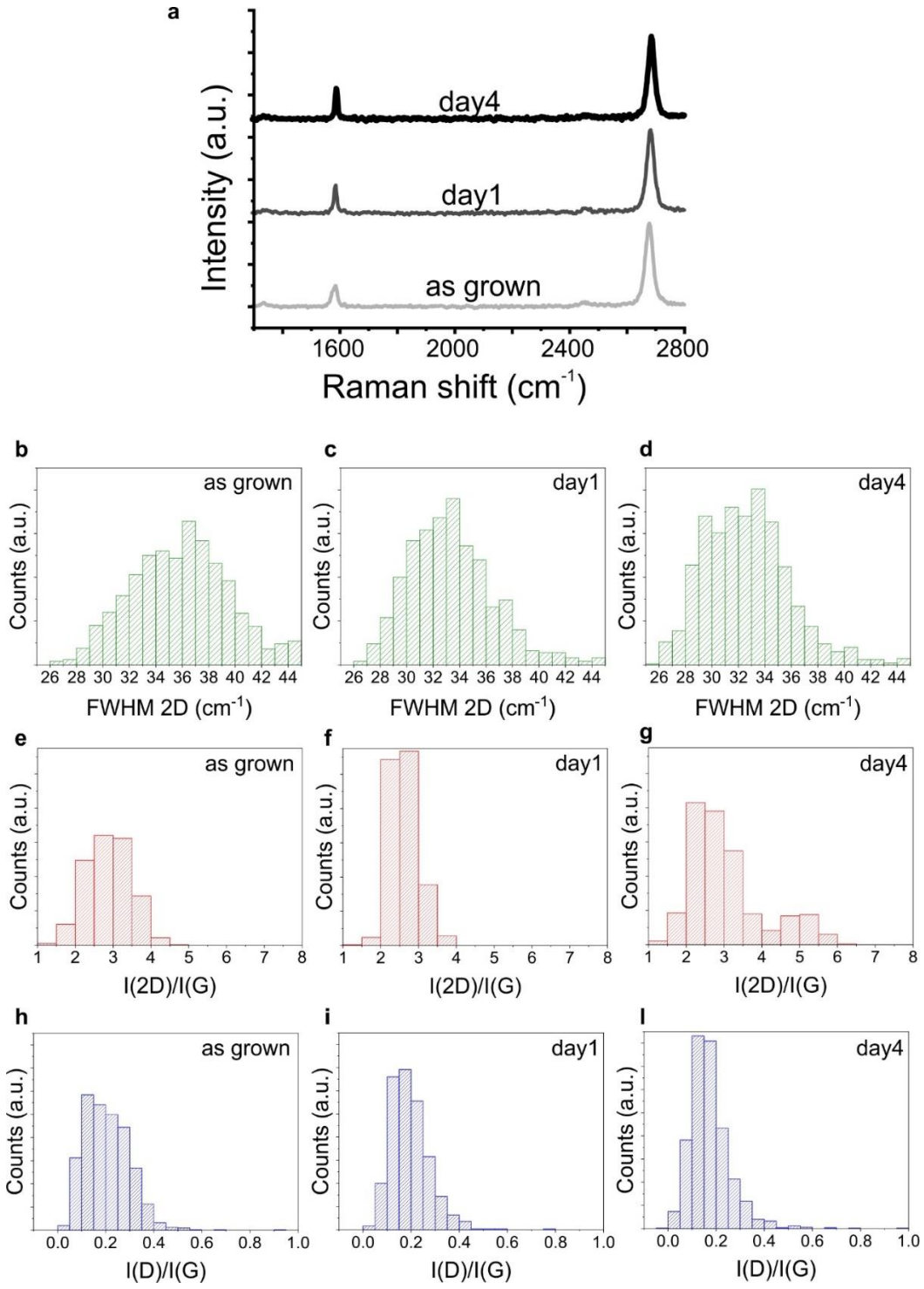

Figure S6. Raman analysis of graphene on sapphire before and after treating with neutrophil-like differentiated HL60 for 1 and 4 days. (a) Characteristic Raman spectra of graphene grown directly on sapphire before and after the incubation with differentiated HL60. (b-h) Representative histograms obtained from 15 × 15 μm^2 Raman maps, showing the (b-d,) FWHM of the 2D peak, (e-g) the 2D/G intensity ratio, and (h-l) the D/G intensity ratio, before (b,e,h) and after 1 day (c,f,i) and 4 days (d,g,l) of incubation.

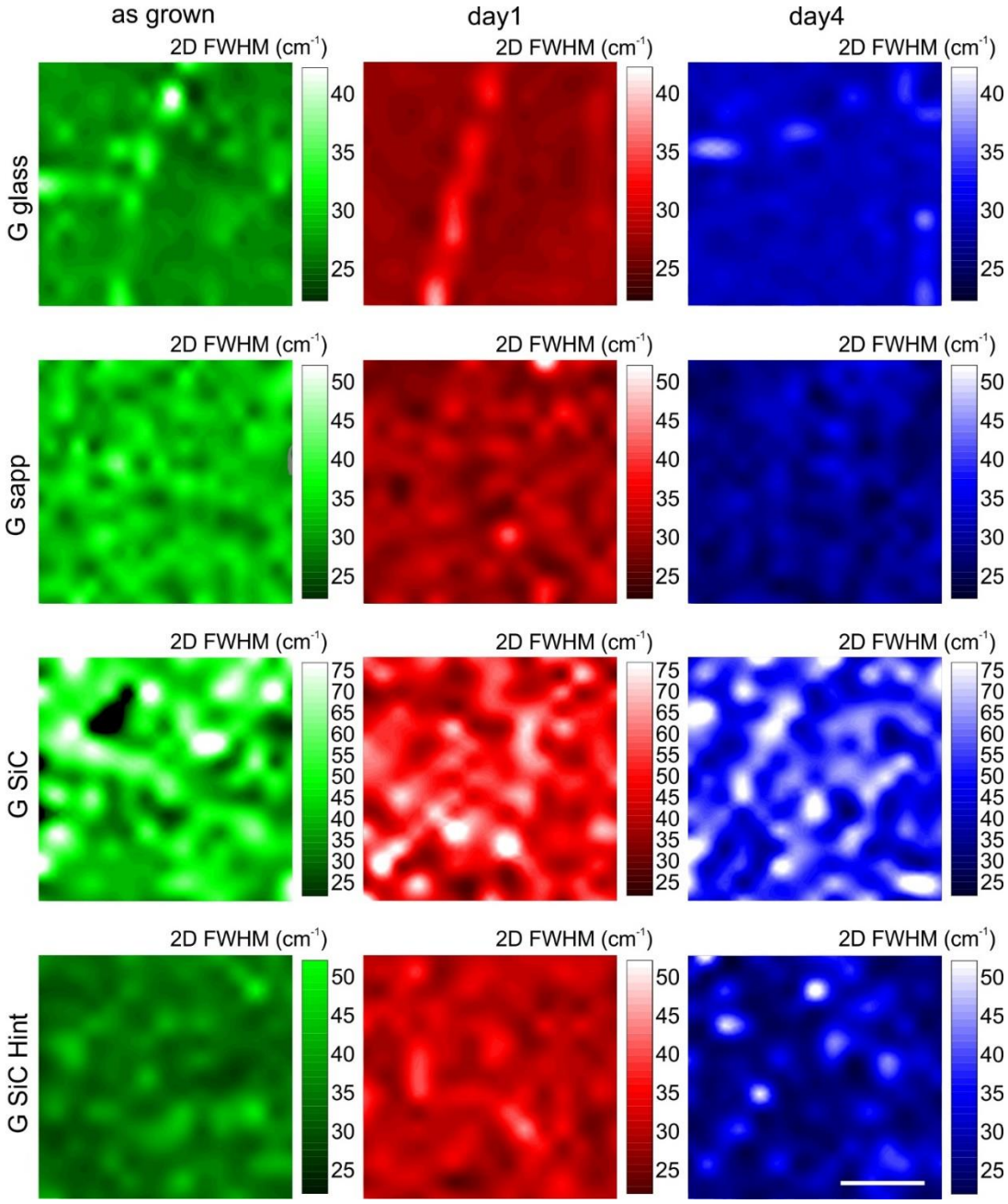

Figure S7. Raman map of the 2D peak FWHM of graphene substrates, before and after 1 day and 4 days of incubation with neutrophil-like differentiated HL60. For all the substrates, the 2D peak width after the incubation shows values close to the as-grown sample values. The distribution of the values is mainly due to the intrinsic variations of the peak width in the area. Scale bar: 5 µm.

Unfortunately, due to the presence of the SiC background, D and G peaks overlapped with the graphene ones, it was not possible to calculate precisely the D/G and 2D/G ratio as we did for graphene on glass (**Figure 3**, in the main text) and graphene on sapphire (**Figure S6**) Nevertheless, no additional D peaks were visible after the incubation, except for those already present after the growth.

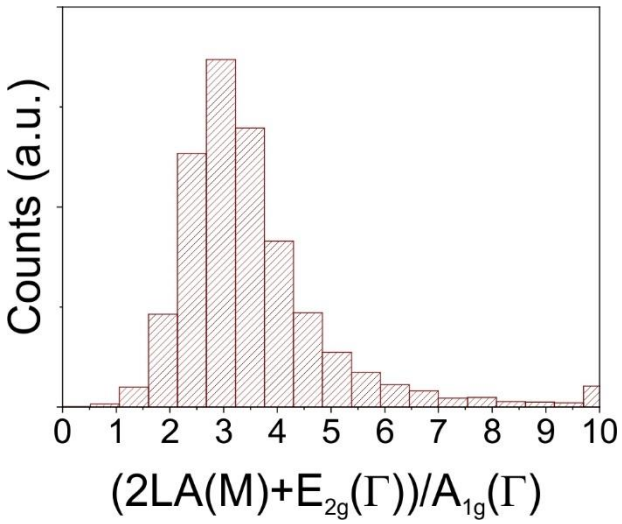

Figure S8. Representative histograms obtained from 15 × 15 μm^2 Raman maps (acquired with a 532 nm green laser), showing the ratio of the 2LA(M) + $E_{2g}(\Gamma)$ and $A_{1g}(\Gamma)$ Raman modes after 4 days of incubation with neutrophil-like differentiated HL60. Raman spectroscopy confirms that WS_2 is monolayer with a high degree of homogeneity, in fact the ratio between the 2LA(M) + $E_{2g}(\Gamma)$ and A(Γ) Raman modes is higher than 2.2, as previously demonstrated in [5,6]. The distribution is peaked at 3.65, comparable with what previously reported in ref.[7], confirming no alternation in the WS_2 samples.

MSC adhesion on 2D materials

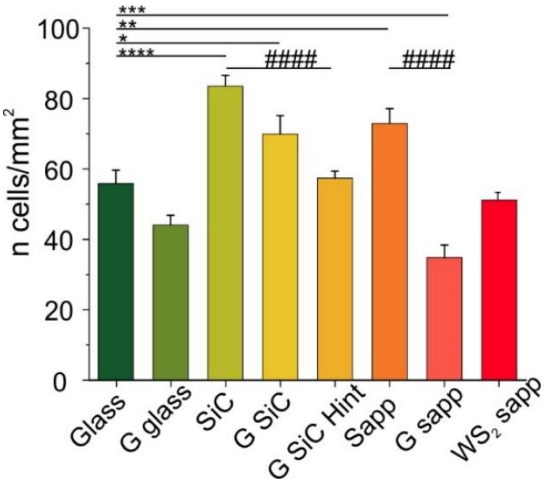

Figure S9. Number of MSCs cultured on 2D materials per 1 mm^2. Data are reported as mean ± s.e.m. of two independent experiments per substrate. One-way ANOVA with Dunnet post hoc test was used for statistical significance with respect to glass control, with *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001, and with Šídák post hoc test for statistical significance with respect to the growth substrate, with ####p < 0.0001.

BDNF expression in MSC

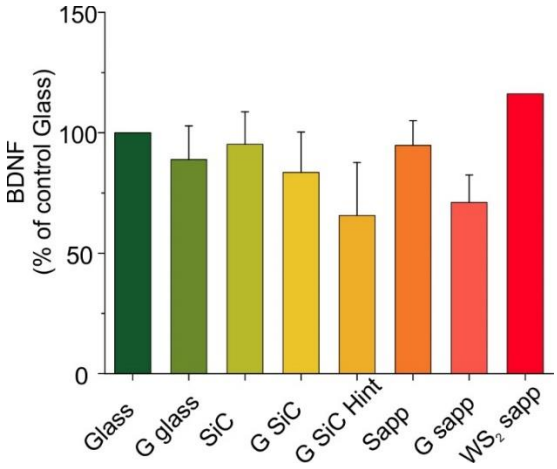

Figure S10. Evaluation of the Brain Derived Neurotrophic Factor (BDNF) expression in MSCs seeded on different substrates. Cells were seeded on the materials for 72 hours and the supernatant was collected, centrifuged, and used to assess the BDNF release. Data are expressed as percentage versus glass control. No statistical significance (One-way with Sidak post hoc test, $p < 0.05$) was retrieved.

Additional AFM analysis on MSC

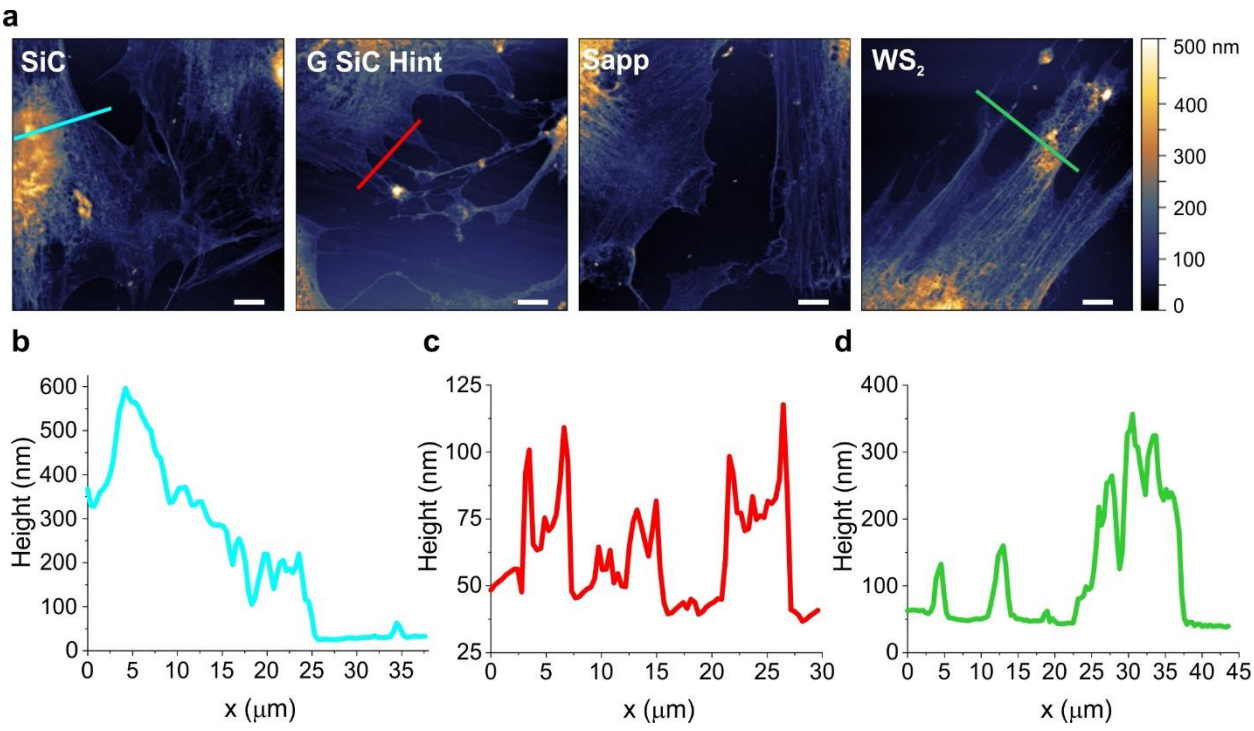

Figure S11. Characteristics of MSC morphology on different substrates. (a) Morphological images of MSC on SiC, hydrogen-intercalated graphene on SiC (G SiC Hint), sapphire (Sapp) and WS_2. Scale bars: 10 µm. (b,c,d) Line profiles along a cell body (b, cyano), and cell protrusions (c, red and d, green). The cells showed a morphology similar to the one of the cells reported in **Figure 5** in the main text.

Raman analysis af WS_2 after MSCs culture

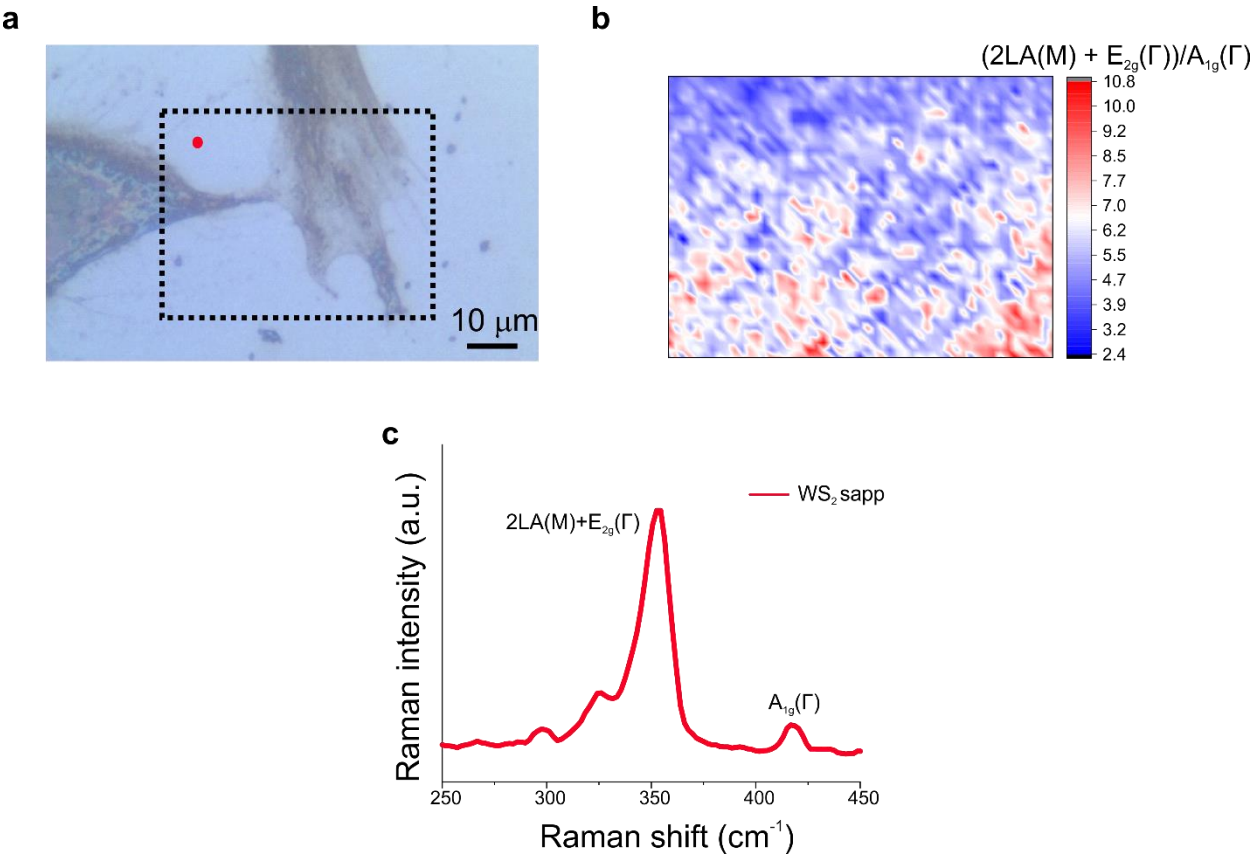

Figure S12. CVD WS_2 stability after MSCs culture. (a) Optical image of MSCs after 4 days of culture on WS_2 with (b) the map of the ratio of the 2LA(M) + $E_{2g}(\Gamma)$ and $A_{1g}(\Gamma)$ Raman modes of the same area. (c) Characteristic Raman spectra of the CVD-WS_2 after MSCs culture (clean area indicated by the red dot in panel (a)). The Raman map confirmed the full coverage of the area also underneath the cell, with a variability mainly ascribable to cell culture residuals.

References

1 D. Convertino, S. Luin, L. Marchetti and C. Coletti, Front. Neurosci., 2018, 12, 1–8.

2 D. Convertino, F. Fabbri, N. Mishra, M. Mainardi, V. Cappello, G. Testa, S. Capsoni, L. Albertazzi, S. Luin, L. Marchetti and C. Coletti, Nano Lett., 2020, 20, 3633–3641.

3 V. Miseikis, D. Convertino, N. Mishra, M. Gemmi, T. Mashoff, S. Heun, N. Haghighian, F. Bisio, M. Canepa, V. Piazza and C. Coletti, 2D Mater., 2015, 2, 014006.

4 N. Mishra, S. Forti, F. Fabbri, L. Martini, C. McAleese, B. R. Conran, P. R. Whelan, A. Shivayogimath, B. S. Jessen, L. Buß, J. Falta, I. Aliaj, S. Roddaro, J. I. Flege, P. Bøggild, K. B. K. Teo and C. Coletti, Small, 2019, 1904906, 1–8.

5 A. Berkdemir, H. R. Gutiérrez, A. R. Botello-Méndez, N. Perea-López, A. L. Elías, C. I. Chia, B. Wang, V. H. Crespi, F. López-Urías, J. C. Charlier, H. Terrones and M. Terrones, Sci. Rep., 2013, 3, 1–8.

6 S. Pace, M. Ferrera, D. Convertino, G. Piccinini, M. Magnozzi, N. Mishra, S. Forti, F. Bisio, M. Canepa, F. Fabbri and C. Coletti, J. Phys. Mater., 2021, 4, 024002

7 D. Convertino, N. Mishra, L. Marchetti, M. Calvello, A. Viegi, A. Cattaneo, F. Fabbri and C. Coletti, Front. Neurosci., 2020, 14, 1–10.