

## Supporting Information

### Simultaneous Drug Delivery and Cellular Imaging Using Graphene Oxide

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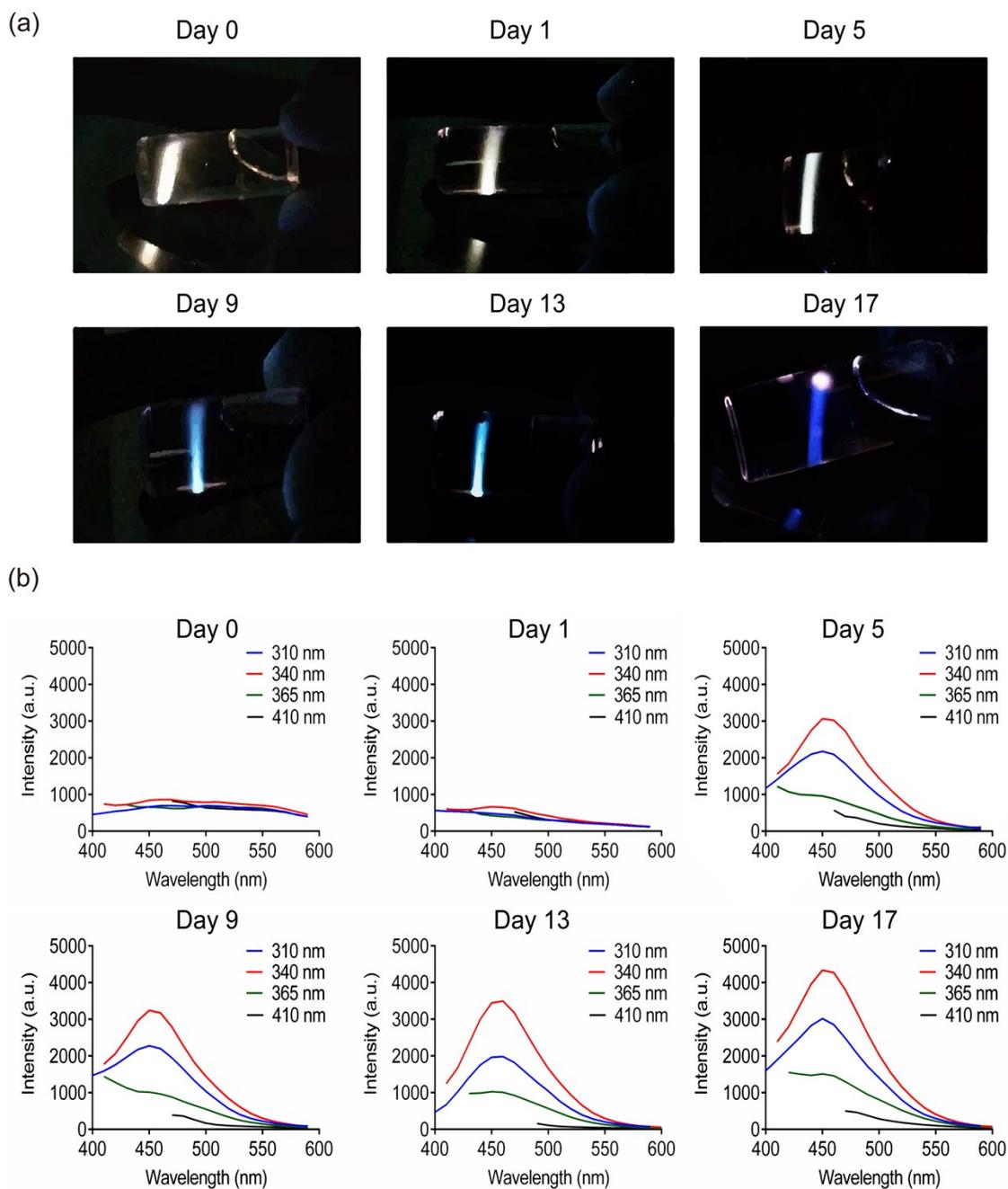
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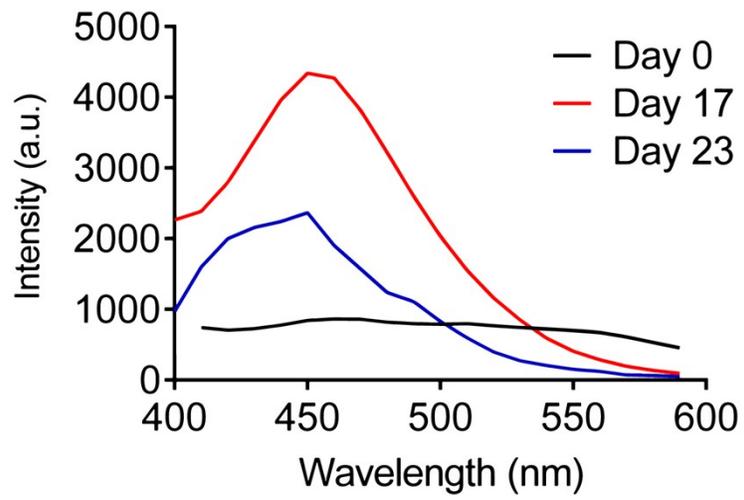
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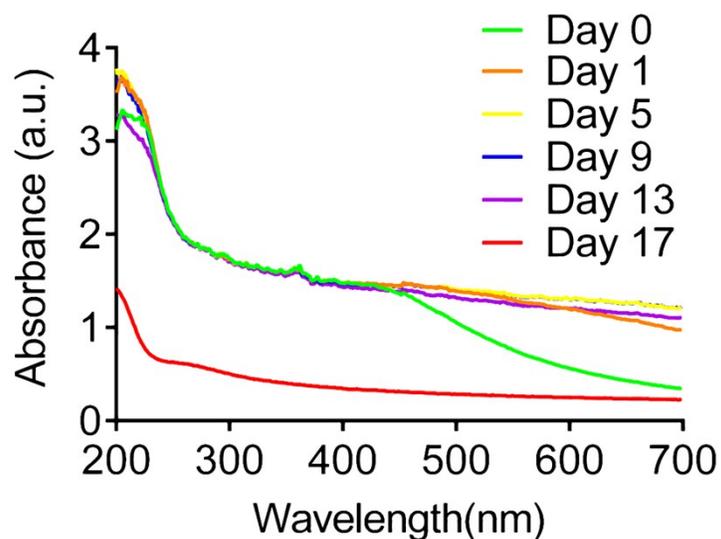
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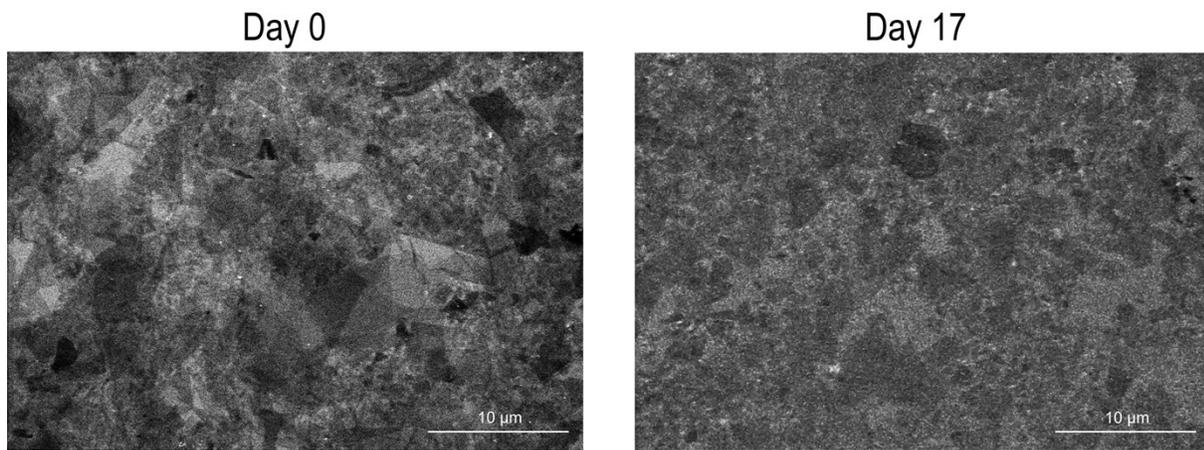
**Figure S1.** (a) Photographs of tunable photoluminescence emission from graphene oxide at annealing times of 0, 1, 5, 9, 13 and 17 days. (b) Photoluminescence spectra of the annealed-GO suspensions taken under 310, 340, 365 and 410 nm UV lamp irradiations.



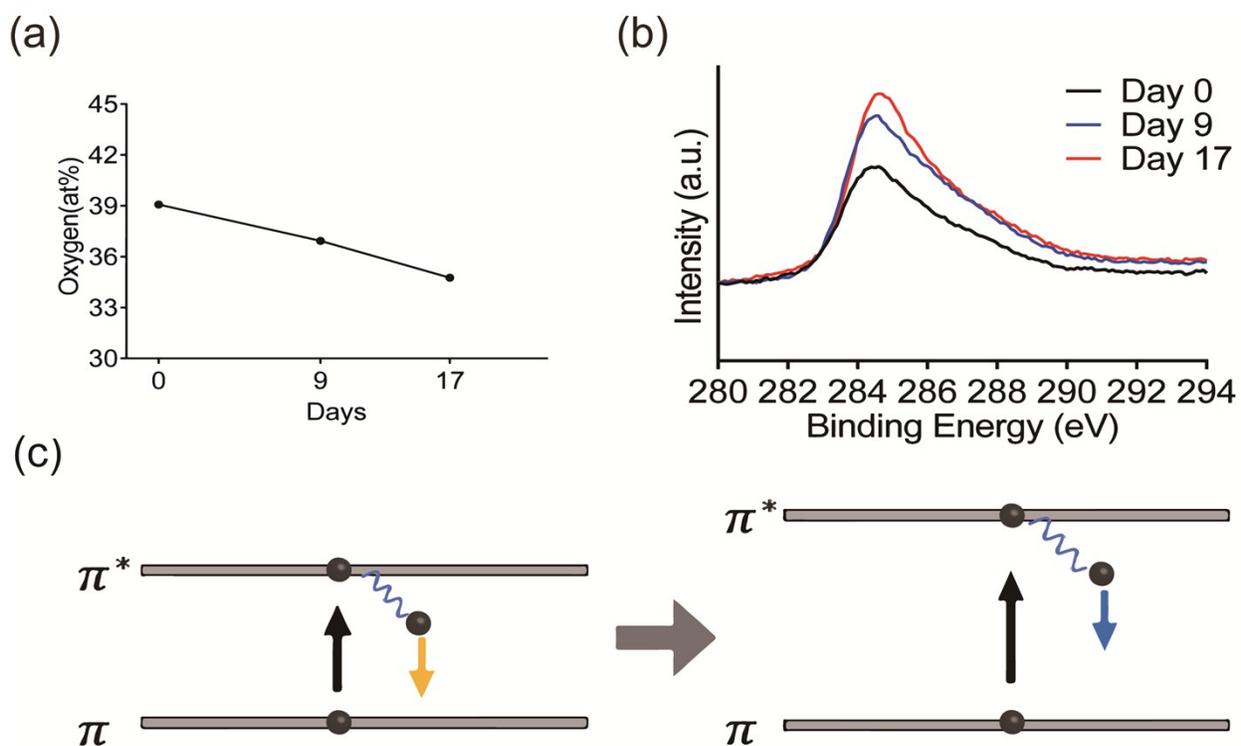
**Figure S2.** Photoluminescence spectra of the annealed-GO (Day 0, Day 17 and Day 23) suspensions taken under 340 nm UV lamp irradiations.



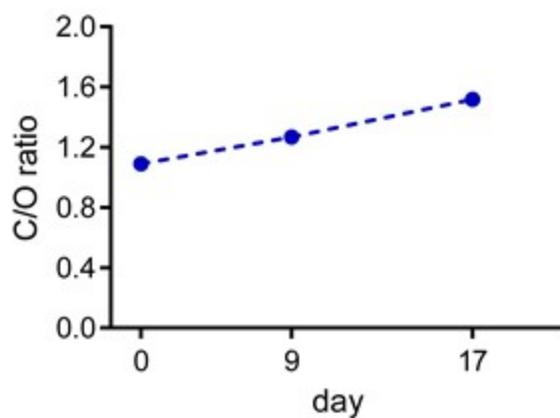
**Figure S3.** UV-VIS absorption spectra from GO at annealing times of 0, 1, 5, 9, 13 and 17 days. Results obtained with UV-VIS spectrophotometry showed that the main absorbance peak at ~230 nm, which is attributed to  $\pi$ - $\pi^*$  transitions of C=C in amorphous carbon systems, remained intact. This result shows that GO sheets do not undergo reduction during mild annealing and is consistent with our previous finding in GO suspensions with thermal annealing.<sup>1-3</sup>



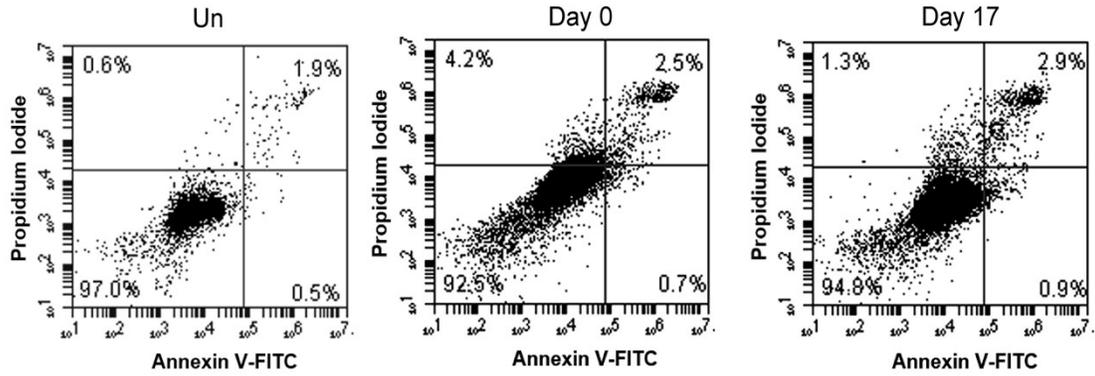
**Figure S4.** SEM images of surface morphology of annealed-GO (Day 17) showed material's thin sheets with even and dense coverage, when compared to the as-synthesized GO samples (Day 0).



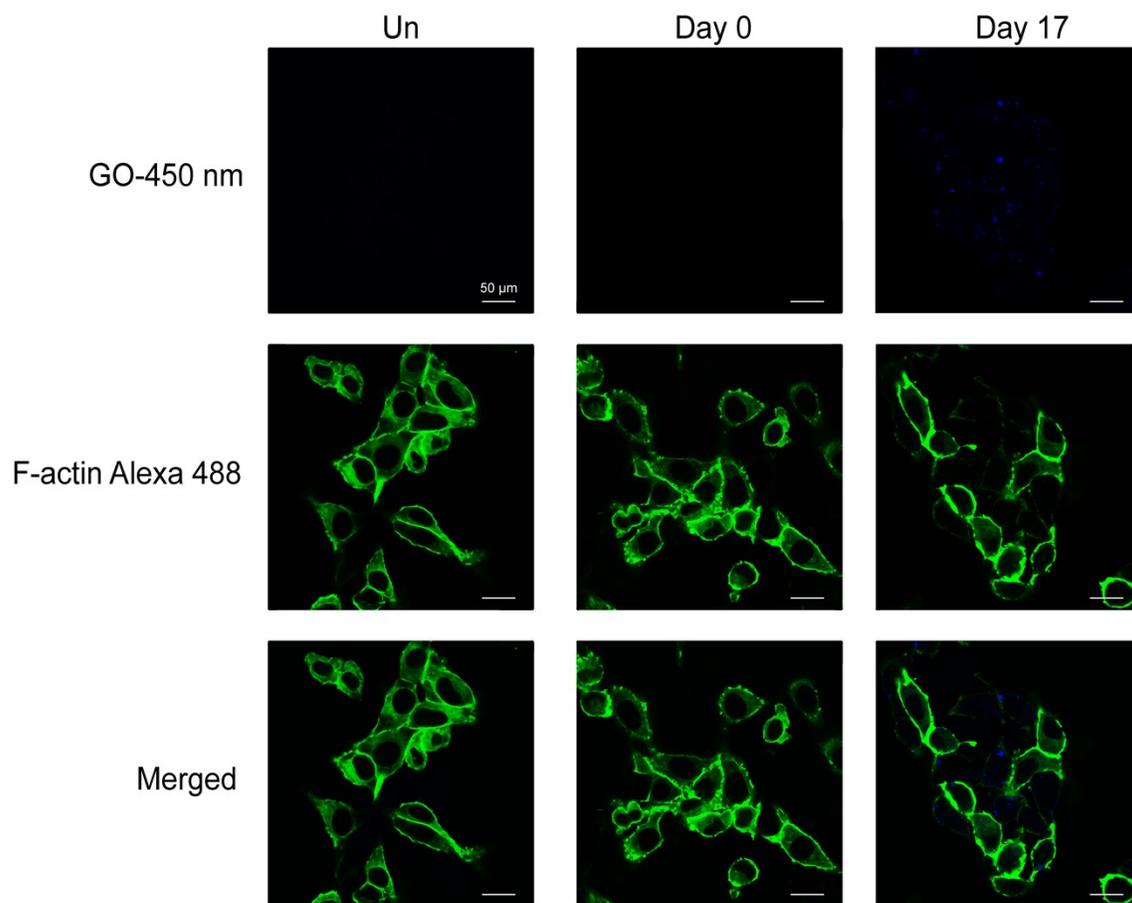
**Figure S5.** The abundance of elements and chemical structure of annealed-GO solutions were investigated quantitatively by XPS analysis. (a) Our results (Fig. S5a and Table S1) show that the oxygen content in annealed-GO Day 9 and 17 had only decreased by 2.15% and 4.32% when compared to the as-synthesized sample (Day 0), clearly indicating that the oxygen functionality is preserved during the annealing process. (b) At the same time, a quantitative analysis of the C1s spectra revealed an increase in the  $sp^2$  C=C bond concentration. (c) Taken together, these results indicate the phase transformation of as-synthesized GO sheets into distinct  $sp^2$  and oxidized domains - with no major loss of oxygen content - in agreement with previous experiments.<sup>1-3</sup>



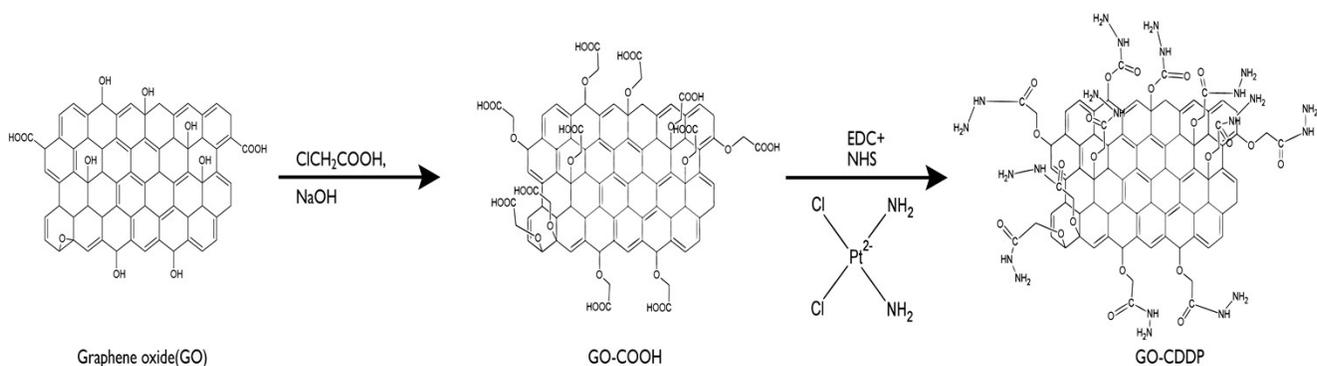
**Figure S6.** According to the XPS spectra shown in Table S1, the C/O ratios of annealed-GO still only slightly (from 1.1 to ~ 1.5 upon annealing, attributed to the loss of water molecules), without significant loss of covalently-bound oxygen content, unlike in the reduction of GO to rGO.<sup>4-6</sup>



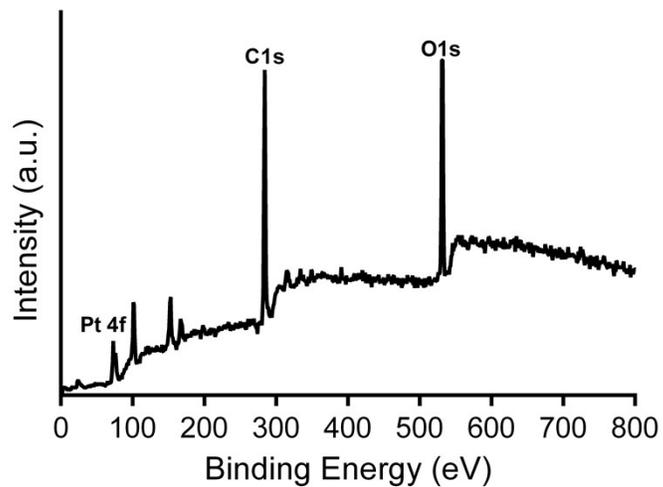
**Figure S7.** Annexin V-FITC and PI staining to evaluate apoptosis in CT26 cells with annealed GO treatment. GO with or without annealing (Day 0 and Day 17)-induced apoptosis in CT26 cells as assayed by flow cytometry. The percentage of early/late apoptotic and necrotic cells are represented in histogram.



**Figure S8.** Confocal microscopy images of the CT26 cells with annealed-GO treatment. Cells were untreated (Un) or treated with 50  $\mu\text{g}/\text{ml}$  GO with or without annealing (Day 0 and Day 17) for 24 h and subjected to filamentous actin (F-actin)-specific immunofluorescence microscopy.



**Figure S9.** To convert the hydroxyl and epoxy groups to carboxyl, GO with or without annealing were dispersed in distilled water, followed by addition of  $\text{ClCH}_2\text{COOH}$  and  $\text{NaOH}$  as described.<sup>7</sup> The carboxylic group on GO is then activated with N-hydroxysuccinimide (NHS) in a reaction catalyzed by 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC). The suspension was sonicated for 30 min at room temperature. CDDP (20 mg) was further added to the suspension and sonicated for another 90 min at room temperature in the dark. The resulting mixture was filtered, washed with PBS and dried at oven for 24 h.<sup>7</sup>



**Figure S10.** The XPS spectrum of GO Day 17-CDDP shows the presence of Pt 4f indicating that CDDP was attached to the GO Day 17 surface, thus provides further evidence of the successful bonding between day 17 sample and CDDP.

**Table S1.** Compositions of different annealed-GO solutions (Day 0, Day 9 and Day 17)

Type (at%)	Day 0	Day 9	Day 17
Si	6.08%	5.17%	5.97%
C	40.93%	45.56%	45.59%
O	39.08%	36.93%	34.76%
N	13.91%	12.34%	13.68%

**Table S2.** Compositions of different annealed-GO solutions with CDDP conjugation

Type (at%)	Day 0	Day 17	Day 0-CDDP	Day 17-CDDP
C	64.21%	61.27%	63.31%	62.12%
O	35.79%	38.73%	33.54%	35.19%
Pt	0%	0%	3.15%	2.68%

## Notes and references

1. N. M. Bardhan, P. V. Kumar, Z. Li, H. L. Ploegh, J. C. Grossman, A. M. Belcher and G.-Y. Chen, *ACS nano*, 2017, **11**, 1548-1558.
2. P. V. Kumar, N. M. Bardhan, G.-Y. Chen, Z. Li, A. M. Belcher and J. C. Grossman, *Carbon*, 2016, **100**, 90-98.
3. P. V. Kumar, N. M. Bardhan, S. Tongay, J. Wu, A. M. Belcher and J. C. Grossman, *Nature chemistry*, 2014, **6**, 151-158.
4. L. Stobinski, B. Lesiak, A. Malolepszy, M. Mazurkiewicz, B. Mierzwa, J. Zemek, P. Jiricek and I. Bielloshapka, *Journal of Electron Spectroscopy and Related Phenomena*, 2014, **195**, 145-154.
5. L. G. Guex, B. Sacchi, K. Peuvot, R. L. Andersson, A. M. Pourrahimi, V. Strom, S. Farris and R. T. Olsson, *Nanoscale*, 2017.

6. S. Pei and H.-M. Cheng, *Carbon*, 2012, **50**, 3210-3228.
7. C. C. Ciobotaru, C. M. Damian, E. Matei and H. Iovu, *Materiale Plastice*, 2014, **51**.