SUPPORTING INFORMATION

Safety Assessment of Graphene-Based Materials: Focus on Human Health and the Environment

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Categorization of graphene-based materials

One important concern in graphene research is that the term "graphene" is used in a generic manner to describe many different graphene-based materials (GBMs). In an attempt to remedy this situation, the Graphene Flagship proposed a classification scheme for GBMs that takes into account three key parameters: the number of graphene layers, the average lateral size, and the carbon-to-oxygen (C:O) atomic ratio.[1] The use of such a classification framework may facilitate the comparison between studies performed in different laboratories and may also enable the assignment of specific physicochemical properties with the safety profile of GBMs.

In the following section, we have prepared 3D plots to illustrate the range of GBMs that have been subjected to toxicological studies, focusing on a few selected areas for which a reasonable number of studies have been published. The first figure (Figure S1) reports the GBMs that have been investigated in the Graphene Flagship in recent years (see Table S1 for details). Most of the studies were conducted on GO of differing lateral dimensions. The second figure focuses on publications dealing with macrophage interactions. The different materials fall into three distinct volumes. Most of them correspond to GO within a narrow range in terms of thickness (number of layers) and carbon/oxygen ratio, while the lateral dimensions cover a broader spectrum (Figure S2). Generally, these GOs all triggering the activation of primary or immortalized macrophages, while the effect on cell viability remains limited, becoming significant only at high concentrations (i.e., 100 μg/mL). The second volume is related to a cluster of materials corresponding to reduced GO and graphene of different size and thickness with a low content of oxygen. The decrease of the number of oxygenated functions on GO surface has a clear impact on the toxic effect of graphene *in vitro* and *in vivo*. The third volume contains four materials with the highest number of layers and the highest lateral size, which correspond to partially exfoliated graphite sheets in the microscale range (erroneously referred to as "nanographites") and to GO with a very high lateral size. Only a low dose-dependent release of proinflammatory cytokines and production of ROS was measured. Cell viability was also not affected, likely because such big sheets are not internalized by the cells and the interactions with the cell membrane does not trigger the same activation of the receptors as found for GO. We also analyzed the publications available on biodegradation of GBMs. The analysis reported in Figure S3 offers a comparative glance of the current landscape around the biodegradation potential of GBMs. The analysis revealed the importance of the surface chemistry of each material. The surface chemistry determines the interaction of GBMs with biological systems. As a consequence, any modification of the surfaces will likely impact their toxic effects and biopersistence. On the other hand, the aqueous colloidal stability of each nanomaterial and the degradation conditions, also play an important role in their (bio)degradability. According to Figure S3, one might conclude that biodegradation is more favorable for the GBMs with a low number of layers and a high number of defects. However, the degradation conditions, such as the type of artificial oxidative method (i.e., photo-Fenton reaction) or the type of enzyme or bacteria, also play a crucial role. In addition, the presence of some gaps regarding the biodegradation of GBMs, mainly due to the lack of examples with different physicochemical characteristics, evidences the urgent need for more research in this area. Figure S4, in turn, shows the studies reported on pulmonary effects of GBMs. The materials range from multi-layered graphene nanoplatelets (GNPs) to atomically thin GO sheets. At present, the majority of studies are concerned with GO and our analysis shows that small lateral dimensions are more frequently associated with toxicological effects. Finally, we have summarized available *in vitro* studies on gastrointestinal effects in Figure S5. The results of this analysis show that GBMs are apparently inert towards intestinal cells, though the relatively small number of studies precludes any wide-ranging conclusions. Overall, as more and more publications are reported, it is important to assign the toxicity profiles of different GBMs with specific physicochemical properties. As discussed in the main text, the density of carbon radicals represents a further parameter to be considered. An improved understanding of the impact of fundamental material properties on the hazard potential of GBMs may aid in safe-by-design approaches to mitigate toxicity while at the same time preserving useful features.

Reference

(1) Wick, P.; Louw-Gaume, A. E.; Kucki, M.; Krug, H. F.; Kostarelos, K.; Fadeel, B.; Dawson, K. A.; Salvati, A.; Vázquez, E.; Ballerini, L.; Tretiach, M.; Benfenati, F.; Flahaut, E.; Gauthier, L.; Prato, M.; Bianco, A. Classification Framework for Graphene-Based Materials. *Angew. Chem. Int. Ed.* **2014**, *53*, 7714-7718.

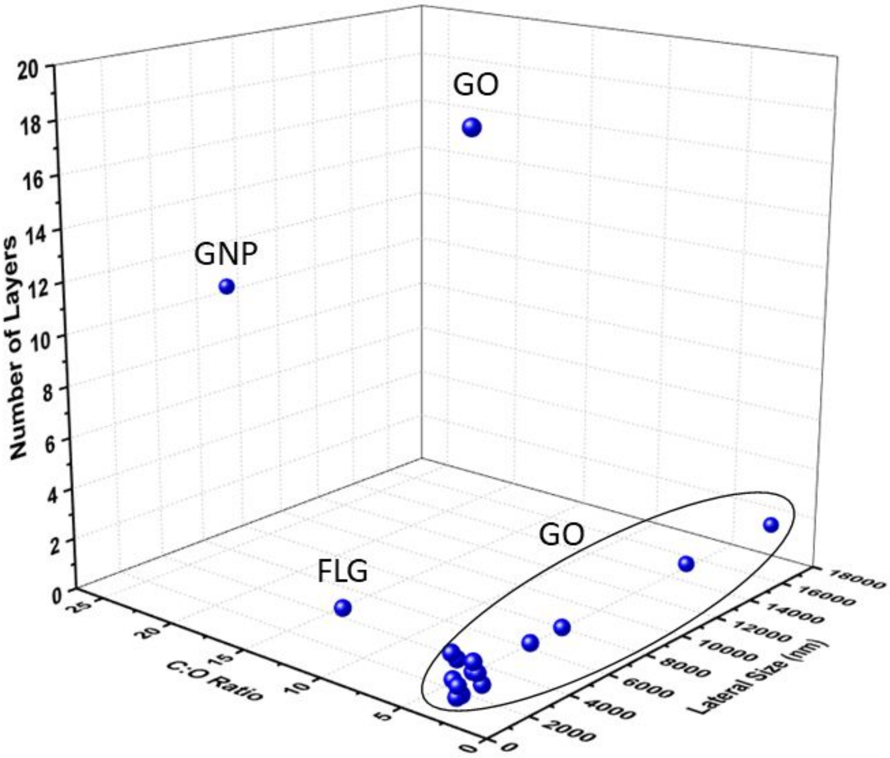

Figure S1. Categorization of the GBMs used in recent studies in the Graphene Flagship according to their three fundamental properties: number of graphene layers, average lateral dimension, atomic carbon/oxygen ratio. Based on: Wick et al. 2014. GO, graphene oxide; FLG, few-layer graphene; GNP, graphene nanoplatelets. GO materials are circled. Refer to Table S1 for details on the publications used to generate this figure.

Table S1. Data and parameters used to generate Figure S1.

Material (commercial source)	Size/lateral dimensions [nm]	Number of layers/thickness	C:O ratio	Ref.
GO GO (Antolin) GO (NanoInnova)	>1000 >1000 400-1000	1-2 1-2 20	1.9 2.17 1.92	R. Kurapati *et al*., *Small* **2015**, 11, 3985
GO 0H* GO 2H GO 26H	1320 270 130	1	1.9 1.9 1.9	J. Russier *et al*., *Nanoscale* **2013**, *5*, 11234
Small GO	50-300	2-3	2.27	M. Orecchioni *et al*., *Nature Comm.* **2017**, *8*, 1109
Large GO Small GO	1000-10000 50-500	1-2 1-2	2.2 2.3	M. Orecchioni *et al*., *Adv. Healthc. Mater*. **2016**, *5*, 276
GO (CheapTubes)	1000-40000	1-4	1.7±0.1	M. Kucki *et al*., *Nanoscale* **2016**, *8*, 8749; M. Kucki *et al*., *J. Nanobiotechnol.* **2017**, 15:46; B. Drasler *et al., Carbon* **2018** [in press]; M. Kucki *et al., 2D Materials* **2018**, *5*, 035014
GO	360±188	1	1.9	M. Kucki *et al*., *Nanoscale* **2016**, *8*, 8749; M. Kucki *et al., 2D Materials* **2018**, *5*, 035014
GO	150±44	1	1.9	M. Kucki *et al*., *Nanoscale* **2016**, *8*, 8749; M. Kucki *et al*., *J. Nanobiotechnol.* **2017**, 15:46; M. Kucki *et al., 2D Materials* **2018**, *5*, 035014
GO (Antolin)	20-1400	1-4	2.61	M. Kucki *et al*., *Nanoscale* **2016**, *8*, 8749; D. Guarnieri *et al., Small* **2018**, 1800227; M. Kucki *et al., 2D Materials* **2018**, *5*, 035014
GNP (CheapTubes)	1000-10000	>10 (20)	24±2.5	M. Kucki *et al*., *Nanoscale* **2016**, *8*, 8749; M. Kucki *et al*., *J. Nanobiotechnol.* **2017**, 15:46; B. Drasler *et al., Carbon* **2018** [in press]
GO-A (Nacional Grafite Ltd., Brasil) GO-B (Nacional Grafite Ltd., Brasil) GO-C (Antolin) GO-D (Graphenea) FLG	10000-15000 15000-20000 85±50 800-7000 630±390	1-2 1-2 1-4 1-2 ≤4	2.4 2.2 0.9 2.2 9.4	S.P. Mukherjee *et al*., PLoS ONE **2016**, *11*, 0166816; D. Guarnieri *et al*., Small **2018**, 1800227; M. Kucki *et al*., 2D Materials **2018**, *5*, 035014

* 0H, 2H, and 26H correspond to the time of sonication of the GO solutions.

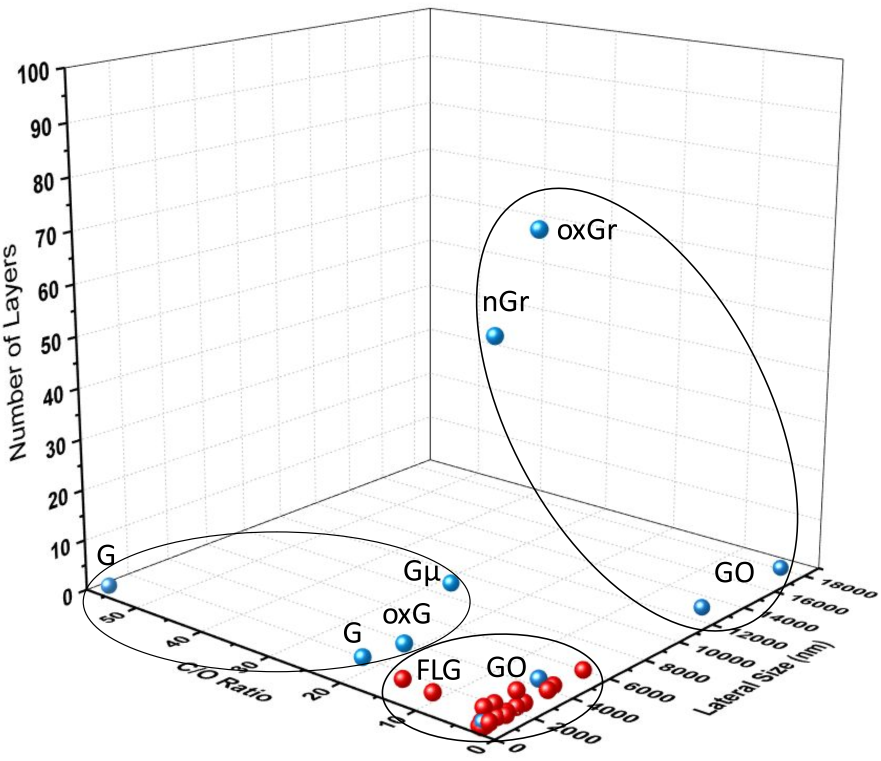

Figure S2. Macrophage studies. Categorization of the different GBMs tested in *in vitro* publications using macrophages according to their three fundamental properties, i.e., number of graphene layers, average lateral dimension, and atomic carbon/oxygen ratio. Note that some properties of GO are not well reported in the literature (e.g., one of the three parameters is sometimes missing), and have been extrapolated considering that Hummers' method was the most used in the synthesis of GO; as the C/O ratio does not vary much from one sample to another, an average value of 1.5 was used. The color codes of the balls highlight the inertness (cyan) and cytotoxicity (red) of the different materials (for further details, refer to the accompanying Table S2).

Table S2. Data and parameters used to generate Figure S2.

Material (commercial source)	Size/lateral dimensions [nm]	Number of layers/thicknes s	C:O ratio	Ref.
FLG	625	3-4	13.3	J. Russier *et al*., *Angew. Chem. Int. Ed.* **2017**, *5*, 11234
Oxidized graphene (oxG)	50-500 (AFM) 250-1750 (TEM)	8.5 nm	14.1	G. F. Erf *et al*., *J. Appl. Toxicol.* **2017**, *37*, 1317
GO 0H* GO 2H GO 26H	1320 270 130	1 1 1	1.9 1.9 1.9	J. Russier *et al*., *Nanoscale* **2013**, *5*, 11234
GO	3000	1	0.78	X. Wu *et al*., *Colloids Surf. B Biointerfaces*, **2017**, *157*, 1
nGO	200	1	2.25	N. Luo *et al*., *Nat. Commun*. **2017**, *8*, 14537
GO	2000	1	2.25	L. A. Visani de Luna *et al*., *J. Nanobiotechnol*. **2016**, 14:12
Small GO Intermediate GO Large GO	50-350 350-750 750-1300	1 1 1	2.25	J. Ma *et al*., *ACS Nano* **2015**, *9* 10498
Nanographite (nGr) Oxidized nanographite (oxGr)	4500 4500	60 80	11.5 6.14	A. Figarol *et al., Toxicol. In Vitro* **2015**, *30*, 476
GO Reduced GO	1100 3500	1 few layers	2.8 3.1	S. A. Sydlik *et al., ACS Nano* **2015**, *9,* 3866
nGO	150	1	2.25	N. Luo *et al*., *ACS Appl. Mater. Interfaces* **2015**, *7*, 5239
GO	450	1	2.25	Y. Li *et al., Small* **2014**, *10*, 1544
Graphene microsheets (Gμ)	4250	10.5	19	Y. Li *et al., PNAS* **2013**, *110*, 12295
GO	475	1	2.25	B. Wan *et al., Toxicol. Lett.* **2013** *22*, 118
GO	1500	1	2.25	G. Qu *et al., ACS Nano* **2013**, *7*, 5732
Large GO Small GO	2400 350	1 1	2.25	G. Y. Chen *et al., Biomaterials* **2012**, *33*, 6559
Large GO Small GO	2000 350	4 4	2.25	H. Yue *et al., Biomaterials* **2012**, *33*, 4013
Pristine graphene (G) Oxidized graphene (oxG)	160 128	2 2	54.87 1.78	A. Sasidharan *et al., Small* **2012**, *8*, 1251
Pristine graphene in 1% pluronic F108	750	4	19	Y. Li *et al., Biomaterials* **2012**, *33* 402
Large GO Small GO	1000-10000 50-500	1-2 1-2	2.2 2.3	M. Orecchioni *et al*., *Adv. Healthc. Mater*. **2016**, *5*, 276
Small GO	50-300	2-3	2.27	M. Orecchioni *et al*., *Nat. Commun.* **2017**, *8*, 1109
Large GO Small GO	10000-40000 50-300	1-2 1-2	2.2 2.3	S. Mukherjee *et al.,* Chem **2018**, 4, 334; S. Mukherjee *et al., Adv. Healthc. Mater*. **2018**, *7*, 1700815
GO-A (Nacional Grafite Ltd., Brasil) GO-B (Nacional Grafite Ltd., Brasil) GO-C (Antolin) GO-D (Graphenea) FLG	10000-15000 15000-20000 85±50 800-7000 630±390	1-2 1-2 1-4 1-2 ≤4	2.4 2.2 0.9 2.2 9.4	S.P. Mukherjee *et al*., PLoS ONE **2016**, *11*, 0166816

* 0H, 2H, and 26H correspond to the time of sonication of the GO solutions.
** For oxidized graphene, GO or rGO, The thickness of about 1 nm corresponds to 1 layer.
*** C/O values not present in the publications were kindly provided by the authors.

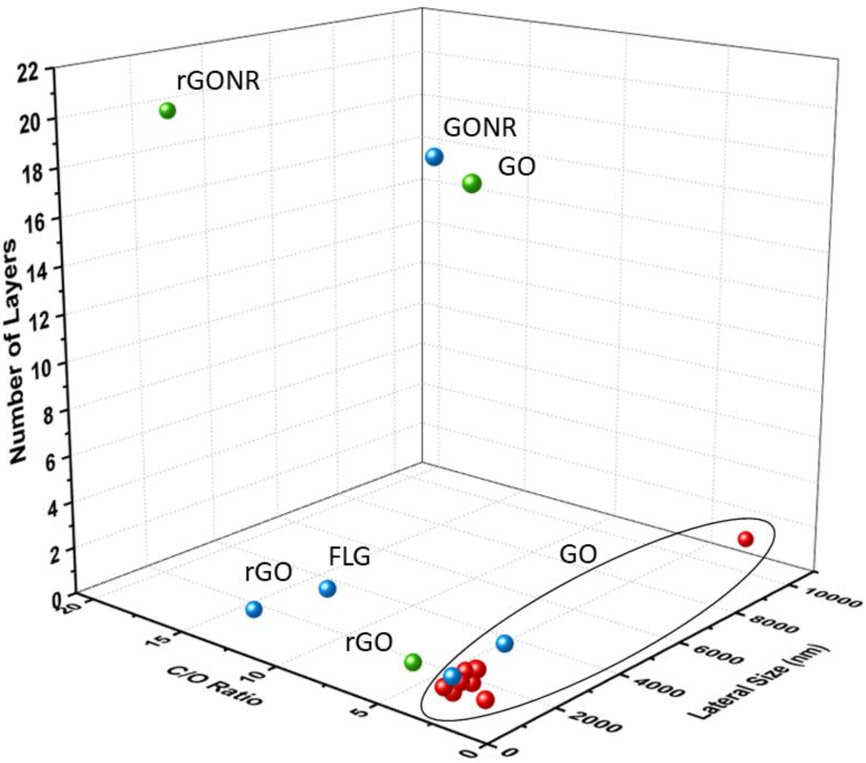

Figure S3. Biodegradation. Categorization of the GBMs tested in studies on degradation reported in the literature according to their three fundamental properties, i.e., number of graphene layers, average lateral dimension, and atomic carbon/oxygen ratio. For detailed discussions of all of these studies, refer to main text. The colour codes of the balls correspond to degradable (red), non-degradable (green) and partially degradable (cyan) materials. The oval circle groups GO materials of different lateral sizes. Refer to Table S3 for details on the publications used to generate this figure.

Table S3. Data and parameters used to generate Figure S3.

Material (commercial source)	Size/lateral dimensions [nm]	Number of layers/ thickness**	C/O ratio***	Oxidation system	Ref.
GO Reduced GO	500-1500 500-1500	0.61 nm 1.73 nm	2.25 5	HRP	G. P. Kotchey *et al., ACS Nano.* **2011**, 5, 2098
GONRs Reduced GONRs	2000 2000	Multi-layer (20) Multi-layer (20)	5.66 19	LiP / VA	G. Lalwani et al., *J. Mater. Chem. B* **2014**, *2*, 6354
GO Reduced GO	476 476	1-2 1-2	1.93 12	Naphthalene-degrading bacteria	L. Liu *et al., Nanoscale* **2015**, *7*, 13619
GO	589±700	0.972 nm	1	P.F. reaction	H. Bai et *al., J. Phys. Chem. C* **2014**, *118*, 10519
GO	400-500	1 nm	2.25	HRP	Y. Li *et al., Small* **2014**, *10*, 1544
GO Reduced GO Graphene (G)	Not given*	Not given	2.09 6.79 10.26	HRP	C. Zhang *et al., Carbon* **2015**, *94,* 531
Oxidized graphene	128	2	1.77	Macrophages	G. M. Girish *et al., Adv. Healthc. Mater.* **2013**, 2, 1489
GO GO (Antolin) GO (NanoInnova)	>1000 >1000 400-1000	1-2 1-2 20	1.9 2.17 1.92	MPO	R. Kurapati *et al., Small* **2015**, *11*, 3985
GO	800-3000	2	2.17	HRP	R. Kurapati *et al., 2D Mater.* **2018**, *5*, 015020
GO	900±500	2.3±1.1 nm	2.4	NaClO	L. Newman *et al., NPJ 2D Mater. Appl.* **2017**, *1*, 39
Large GO Small GO	10000±8000 100±50	1-2 nm 1-2 nm	2.1 2.1	MPO/Primary human neutrophils	S. P. Mukherjee *et al., Nanoscale* **2018**, *10*, 1180

* This example cannot be inserted in the 3D plot because of the missing data on size and thickness.
** For oxidized graphene, GO or rGO, The thickness of about 1 nm corresponds to 1 layer.
*** C/O values not present in the publications were kindly provided by the authors.

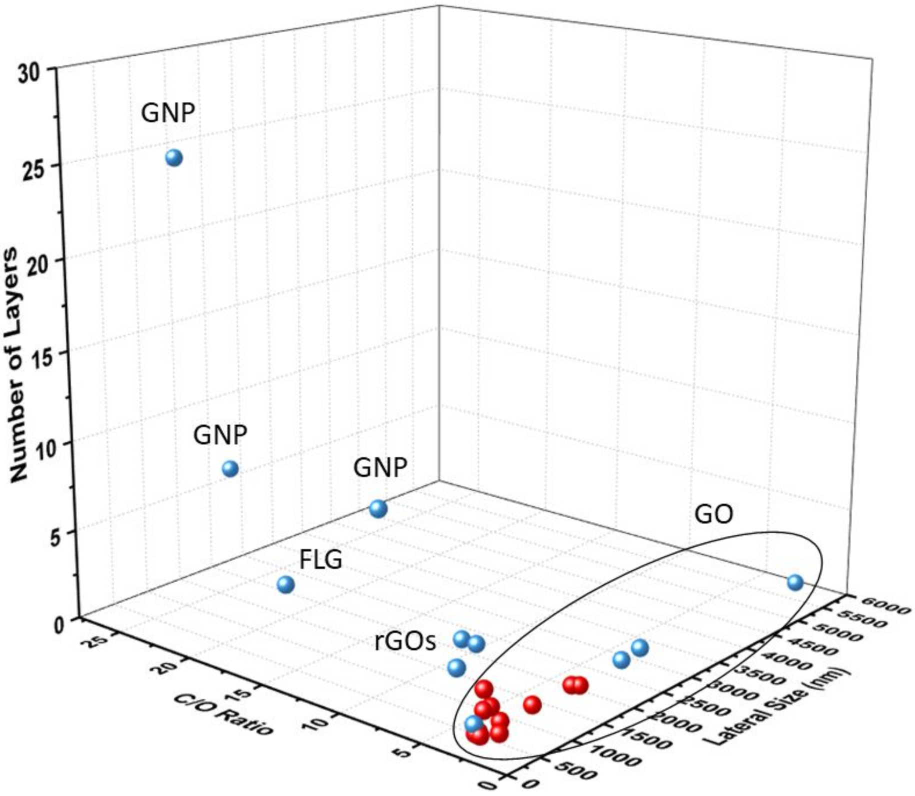

Figure S4. Pulmonary studies. Categorization of the GBMs tested in studies *in vitro* and *in vivo* on pulmonary impact reported in the literature according to their three fundamental properties, i.e., number of graphene layers, average lateral dimension, and atomic carbon/oxygen ratio. The colour codes of the balls correspond to materials that are inflammogenic, provoke lung injuries and granulomas (red), and materials that do not show sign of inflammation or cellular responses (cyan). For detailed discussions of all of these studies, refer to main text. See Table S4 for details on these publications.

Table S4. Data and parameters used to generate Figure S4.

Material	Size/lateral dimensions [nm]	Number of layers/thickness	C:O ratio	Ref.
GO	300	2	2.5	A. Boucetta *et al., Adv. Healthcar. Mater*. **2013**, *2*, 433
FLG	325	5	14.8	L. Mao *et al., Part. Fibre Toxicol.* **2016**, 13:7
GNP	550	10	9.8	J. H. Shin *et al*., *Nanotoxicology* **2015**, *9*, 1023
GNP	1000	25	25	J. K. Kim *et al., Nanotoxicology* **2016**, *10*, 891
GO	405	2	2.5	B. Li *et al., NPG Asia Mater*. **2013**, *5*, e44
GO	158	1.25 nm	2.5	M. C. Duch *et al., Nano Lett.* **2011**, *11*, 5201
GO	2500	2	1.4	S. Bengtson *et al., PLoS One* **2017**, *12*, e01783552017
rGO	1500	2	8.5	
GO	2500	2	1.4	S. Bengtson *et al., Environ. Mol. Mutagen*. **2016**, *57*, 469
rGO	1500	2	8.5	
rGO	1500	2	7.6	
GO	2510	1	2.5	M. R. Shurin *et al., ACS Nano* **2012**, *8*, 5585
GO	225	1	2.5	J. Ma *et al., ACS Nano* **2015**, *10*, 10498
GO	550	1	2.5	
GO	1050	1	2.5	
GO	1676	1	2.5	X. Wang *et al., ACS Nano* **2015**, *9*, 3032
GO	179	1	2.5	
GO	170	1	2.2	S. Vranic *et al.,ACS Nano* **2018**, *12*, 1373
GO	1723	1	2.2	
GO	334	1	1.72	R. Li *et al., ACS Nano* **2018**, *12*, 1390
hydrated GO (hGO)	330	3	2.60	
rGO	549	3	5.06	
GO	1000-10000	1	1.7	B. Drasler *et al., Carbon* **2018** [in press]
GNP	1000-2000	5-10	24	

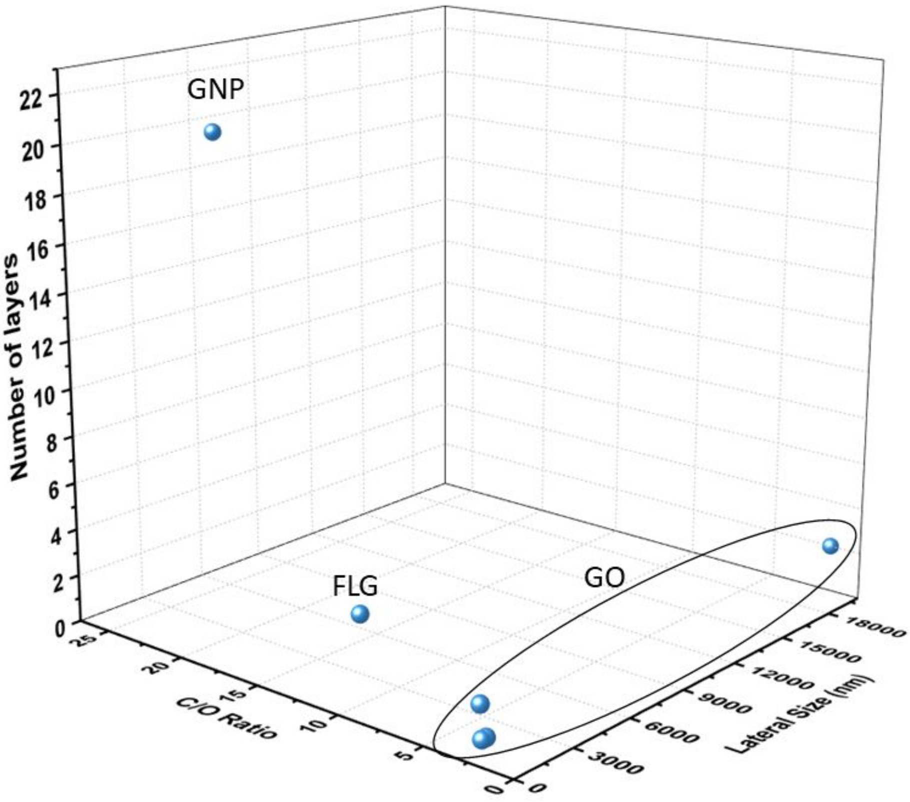

Figure S5. Gastrointestinal studies. Categorization of the GBMs tested in studies *in vitro* using intestinal cells reported in the literature according to their three fundamental properties, i.e., number of graphene layers, average lateral dimension, and atomic carbon/oxygen ratio. The cyan color of the balls refers to the relative inertness of all materials represented in the 3D plot, which trigger a cellular response only at the highest doses. For detailed discussions of these studies, refer to main text, and Table S5.

Table S5. Data and parameters used to generate Figure S5.

Material (commercial source)	Size/lateral dimensions [nm]	Number of layers/thickness	C:O ratio	Ref.
GO (CheapTubes)	1000-40000	1-4	1.7±0.1	M. Kucki *et al., Nanoscale* **2016**, *8*, 8749; M. Kucki *et al., J. Nanobiotechol.* **2017**, 15:46
GO	360±188	1	1.9	M. Kucki *et al., Nanoscale* **2016**, *8*, 8749
GO	150±44	1	1.9	M. Kucki *et al., Nanoscale* **2016**, *8*, 8749; M. Kucki *et al., J. Nanobiotechol.* **2017**, 15:46
GO (Antolin)	20-1400	1-4	2.61	M. Kucki *et al., Nanoscale* **2016**, *8*, 8749; D. Guarnieri *et al., Small* **2018**, 1800227
GNP (CheapTubes)	1000-10000	>10 layers (20)	24±2.5	M. Kucki *et al., Nanoscale* **2016**, *8*, 8749; M. Kucki *et al., J. Nanobiotechol.* **2017**, 15:46
FLG	630±390	4	9.4	D. Guarnieri *et al., Small* **2018**, 1800227