Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information for

Electrostatic self-assembled graphene oxide-collagen scaffolds towards a three-dimensional microenvironment for biomimetic applications

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Hydrogel formation testing

Figure S1 describes the influence of the medium pH and the collagen/GO w/w ratio relatively to the gelation process. It is observable that only a few GO-Col samples have passed the tube inversion test and therefore were considered to be consistent and stable hydrogels. It is also noticed that the collagen % needed to crosslink the GO sheets decreases with the increasing pH.

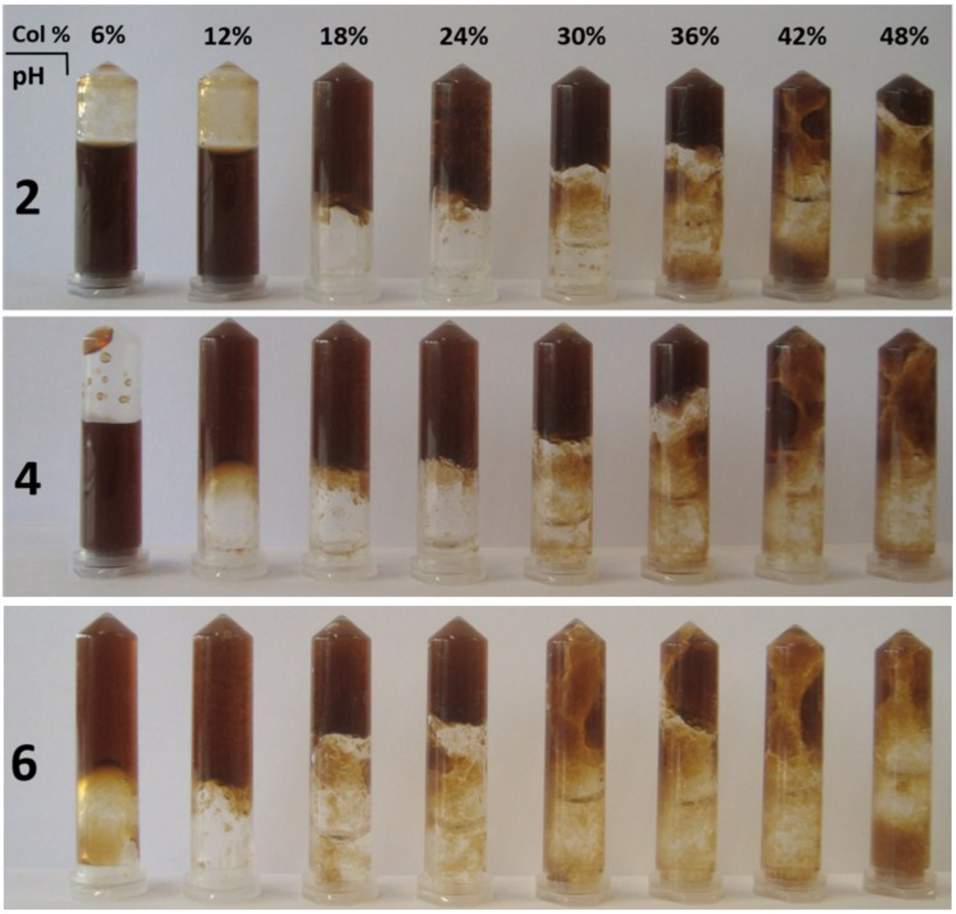

Figure S1. Photographs of 4 mg/mL GO solutions mixed and shaken with collagen at different weight ratios and pH values: 2; 4 and 6. From left to right, Col/GO (w/w %) = 6, 12, 18, 24, 30, 36, 42 and 48.

XPS analysis

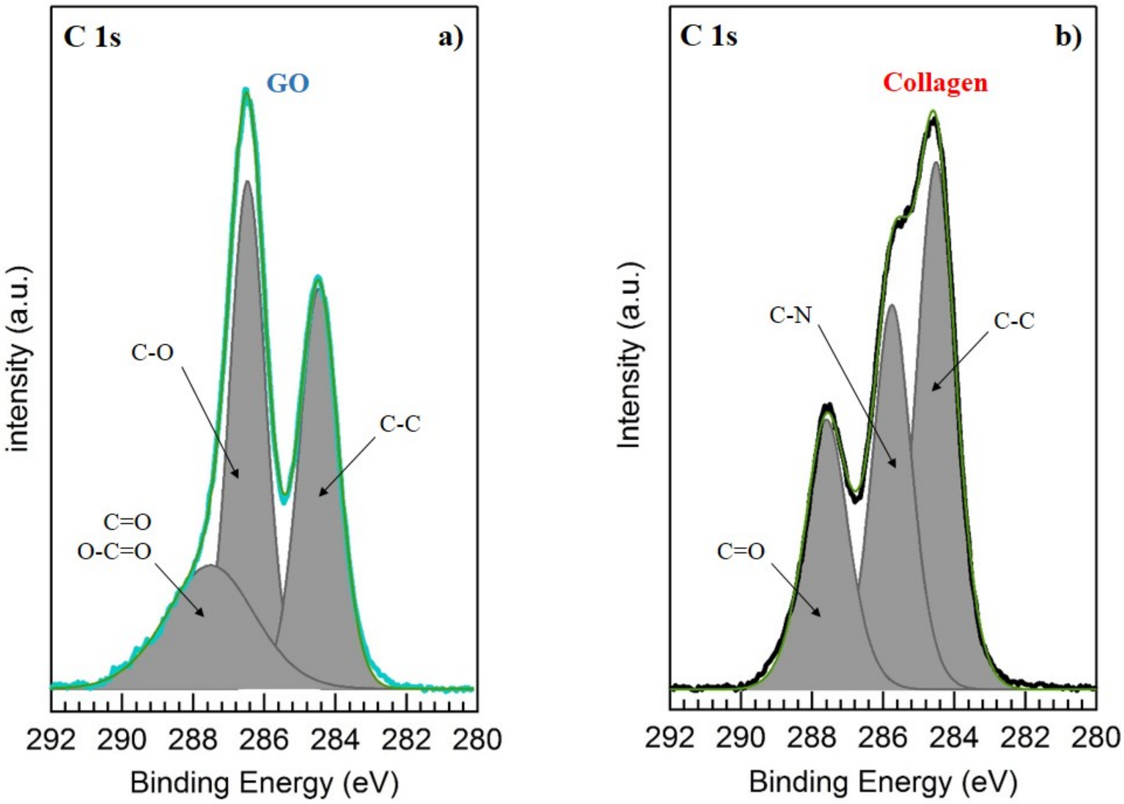

Figure S2. Normalized C1s core levels obtained for a) GO and b) collagen.

Table S 1. Elemental composition of GO and collagen samples obtained by XPS.

	Functional group	BE (eV)	FWHM (eV)	at. %
GO	C-C	284.5	1.3	35
	C-O	286.5	1.2	41
	C=O and O-C=O	287.5	2.9	24
Collagen	C-C	284.5	1.3	44
	C-N	285.7	1.4	32
	C=O	287.6	1.3	24

AFM friction tests

Relatively to the AFM friction tests, the nominal adhesion energy (W_{adh}) can be obtained from the adhesion force (F_{adh}) between a sphere and a flat surface by the Maugis–Dugdale theory $W_{adh} = F_{adh}/(\lambda \pi R_{tip})$, by assuming that both surfaces are ideal (without roughness).[1] In case of SiO_2 cantilever tip λ is equal to 1.66[2] and R_{tip} is equal to 10nm (PPP-CONTR, Nanosensors). In our studies it was observed higher adhesion force for collagen at GO surface than for pure collagen, 0.132 Jm^2 and 0.045 Jm^2 respectively (Table S2). These results showed a stronger interaction between collagen molecules and SiO_2 cantilever tip that could be attributed to the higher induced ordering[3] or even functionalization[4] of molecular collagen at GO surface.

Table S 2. Friction coefficient (K_{fri}), adhesion force (F_{adh}) and adhesion energy (W_{adh}) for GO, Collagen and Go-Col obtained from the respective Frictional versus load curves.

	k_{fric}	F_{adh} (nN)	W_{adh} (J m^2)
GO	0.22	5.7	0.109
Collagen	0.02	2.38	0.046
GO-Col	0.13	6.9	0.132

Swelling tests

Figure S3 shows how the pH of the medium and the % of Col used during the GO-Col hydrogel synthesis affected the swelling ratio of the GO-Col scaffolds. It is observable that the swelling equilibrium is achieved in the first hour of MilliQ water immersion. The compressive moduli of the GO-Col scaffolds at dry and wet states were determined by analysing the stress-strain curves (Fig. S4, S5 and S6). The final results are presented in Fig S7, where it is possible to observe not only the effect of the pH and the % Col present into the system, but also the influence of the water uptake on the mechanical properties of each GO-Col scaffold.

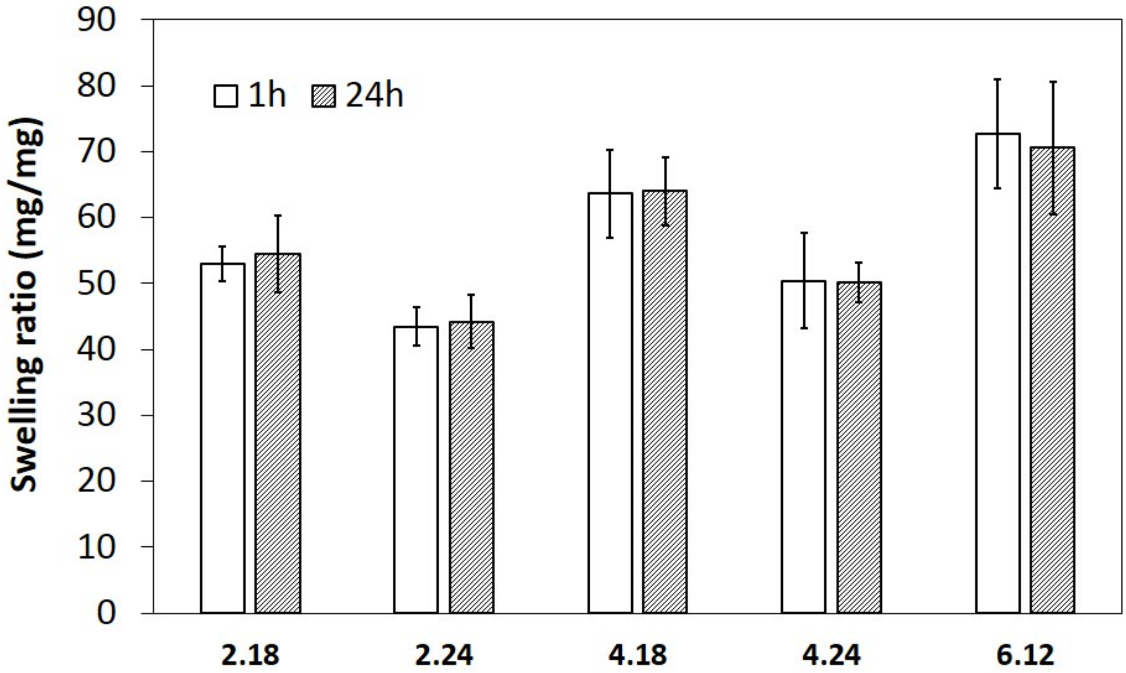

Figure S 3. Swelling ratio of the GO-Col scaffolds after 1h and 24h.

Compressive stress-strain curves of the GO-Col scaffolds

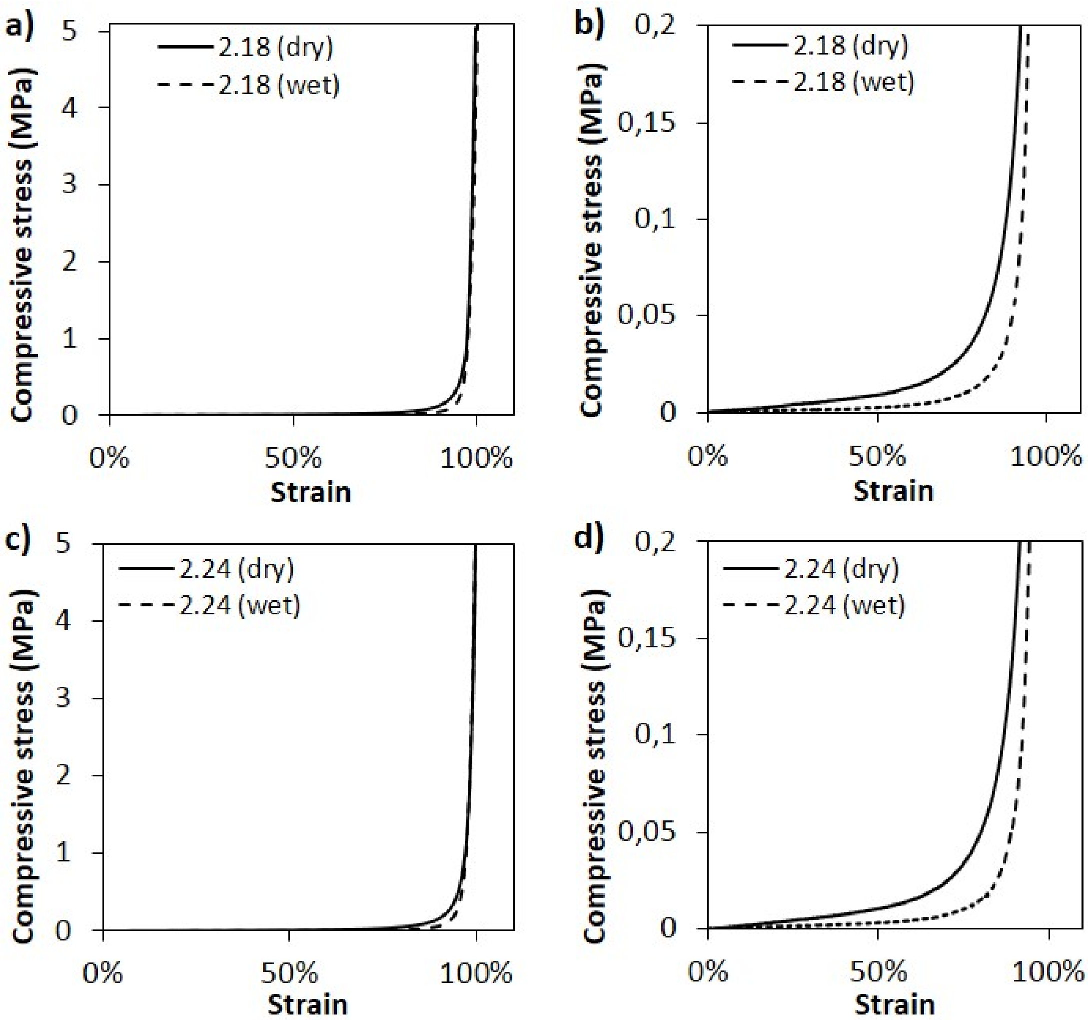

Figure S4. Compressive stress-strain curves of the GO-Col scaffolds at pH 2. **a-b**) 18% of collagen/GO w/w ratio; **c-d**) 24% of collagen / GO w/w ratio.

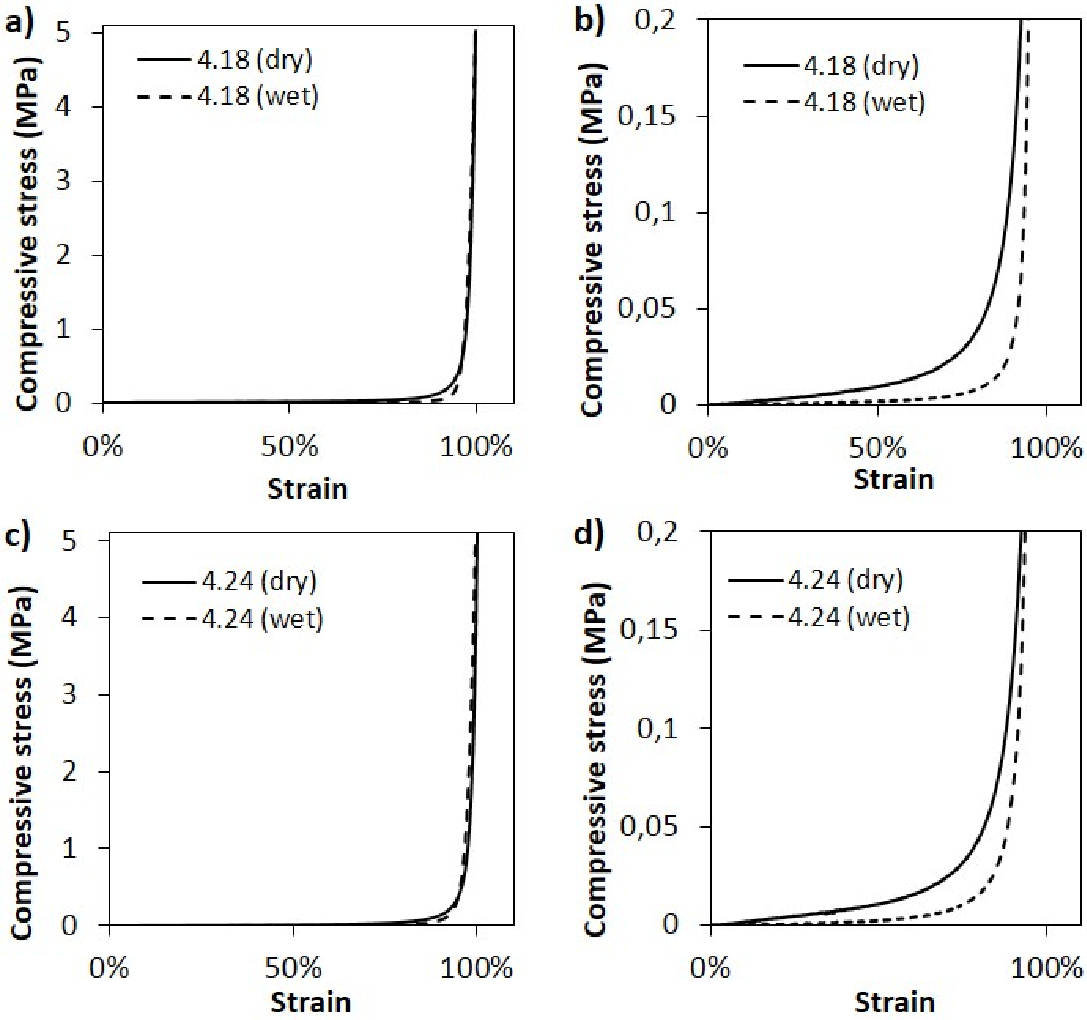

Figure S5. Compressive stress-strain curves of the GO-Col scaffolds at pH 4. **a-b**) 18% of collagen/GO w/w ratio; **c-d**) 24% of collagen / GO w/w ratio.

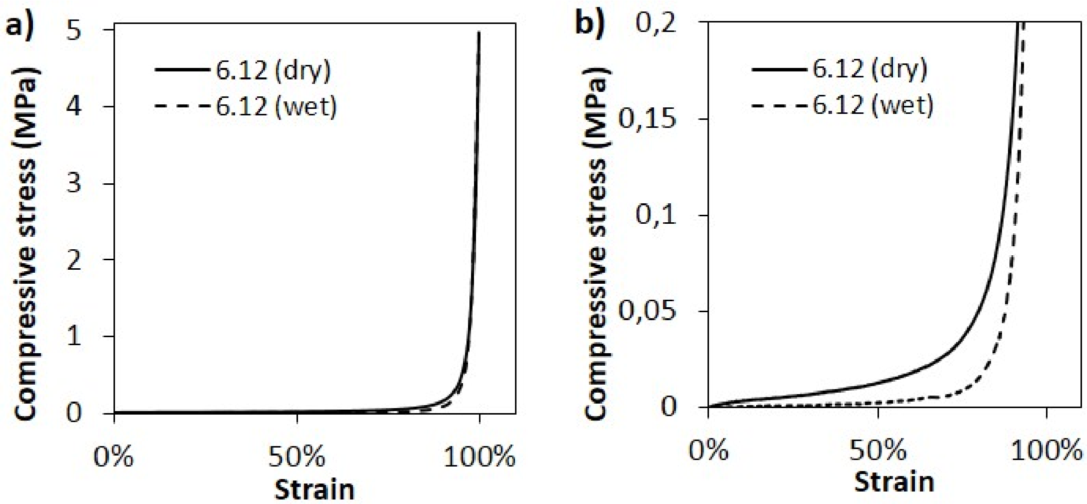

Figure S6. Compressive stress-strain curves of the GO-Col scaffolds at pH 6. **a-b**) 18% of collagen/GO w/w ratio.

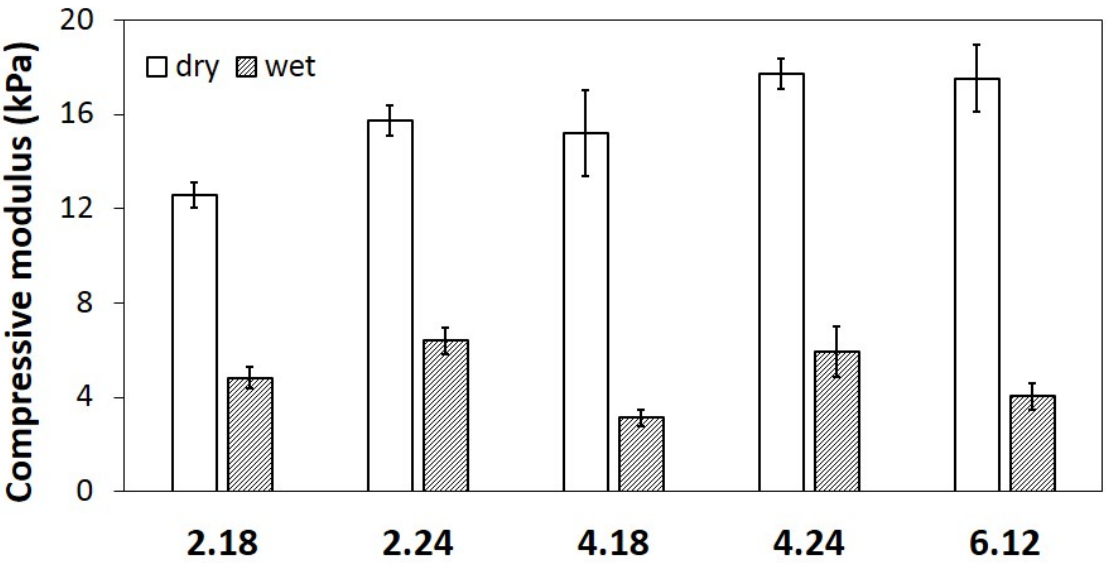

Figure S7. Comparison of the compressive moduli of the GO-Col scaffolds at the dry and wet states.

Quantitative analysis using XPS

Table S 3. Elemental composition of rGO-Col and GO-Col samples obtained by XPS.

Sample	C1s fit				C1s / Os1 ratio	C1s / N1s ratio
	Functional group	**BE (eV)**	**FWHM (eV)**	**at. (%)**		
rGO-Col	C Sp^2	284.5	0.8	34	5	9.6
	C Sp^3 / C-N	285.1	1.3	30		
	C-O	286.2	1.3	23		
	C=O	288.0	1.6	13		
GO-Col	C Sp^2 / C Sp^3 / C-N	284.5	2.6	65	2.4	9.4
	C=O / C-O	286.5	2.1	35		

Swelling and compression tests of GO-Col and rGO-Col

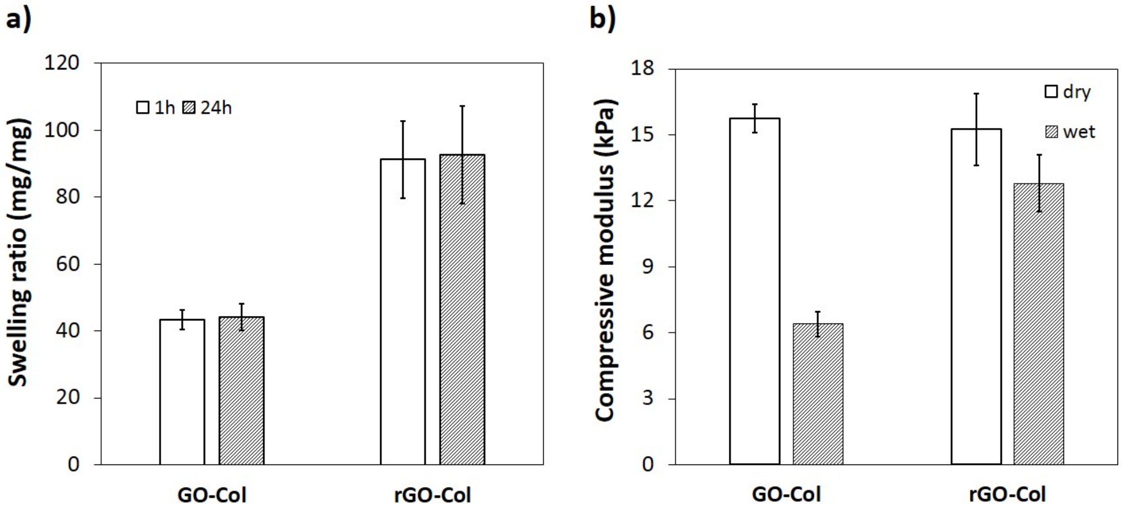

Figure S 8. Comparison of a) the swelling ratio and b) compressive modulus of the GO-Col and rGO-Col.

References:

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2. R. W. Carpick, D. F. Ogletree and M. Salmeron, *J. Colloid Interface Sci.*, 1999, **211**, 395-400.
3. Y. Shin, J. Lee, L. Jin, M. Kim, Y.-J. Kim, J. Hyun, T.-G. Jung, S. Hong and D.-W. Han, *Journal of Nanobiotechnology*, 2015, **13**, 21.
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