

Supplementary information

Graphene induces spontaneous cardiac differentiation in embryoid bodies

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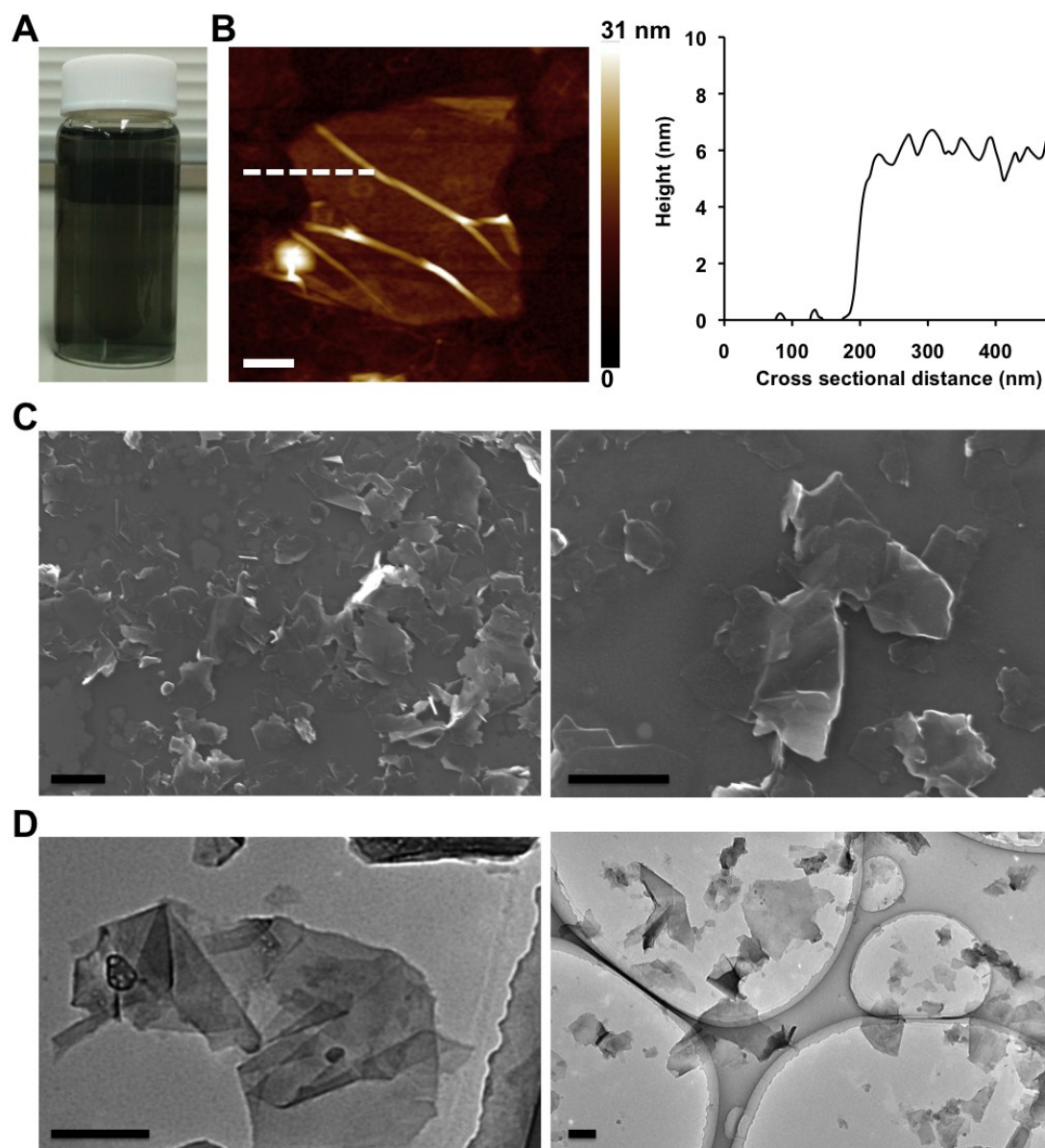


Figure S1. Structural properties of Graphene. (A) Image of 0.2 mg/mL aqueous graphene dispersion. (B) AFM image of a graphene sheet and its corresponding height profile along the dashed line. The scale bar shows 200 nm. (C) Two scanning electron microscopy images of graphene. The scale bars show 1000 nm. (D) Two TEM images of graphene. The scale bars show 200 nm.

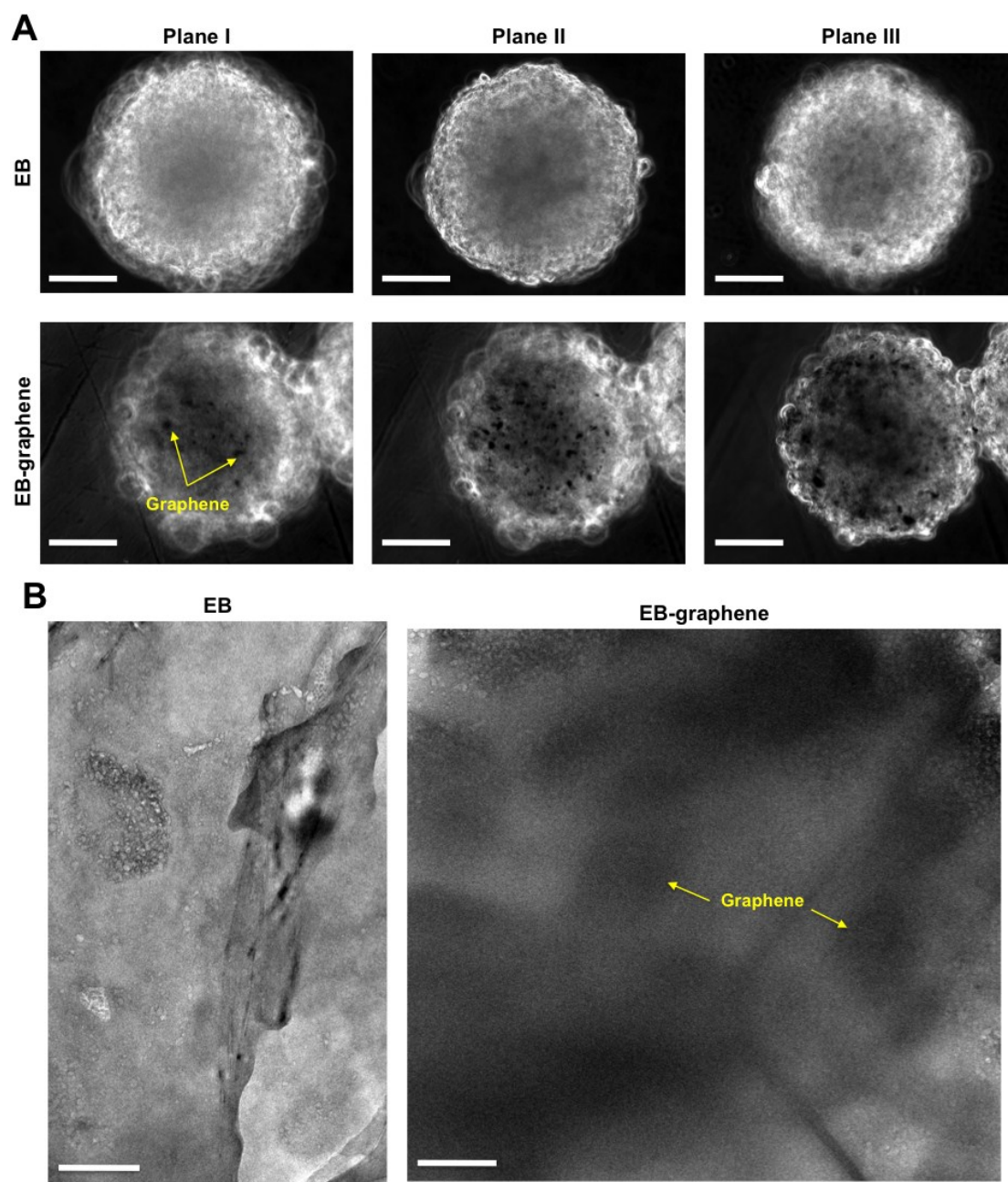


Figure S2. Graphene in the EB structure. (A) Phase contrast images of EB and EB-graphene were focused and taken at different planes along the z-axis after 3 days of culture. The graphene is apparent as dark spots in the EB-graphene. The scale bars show 100 μm . (B) TEM images of EB and EB-graphene after 3 days of culture. The graphene appears as dark areas in the EB-graphene. The scale bars show 200 nm.

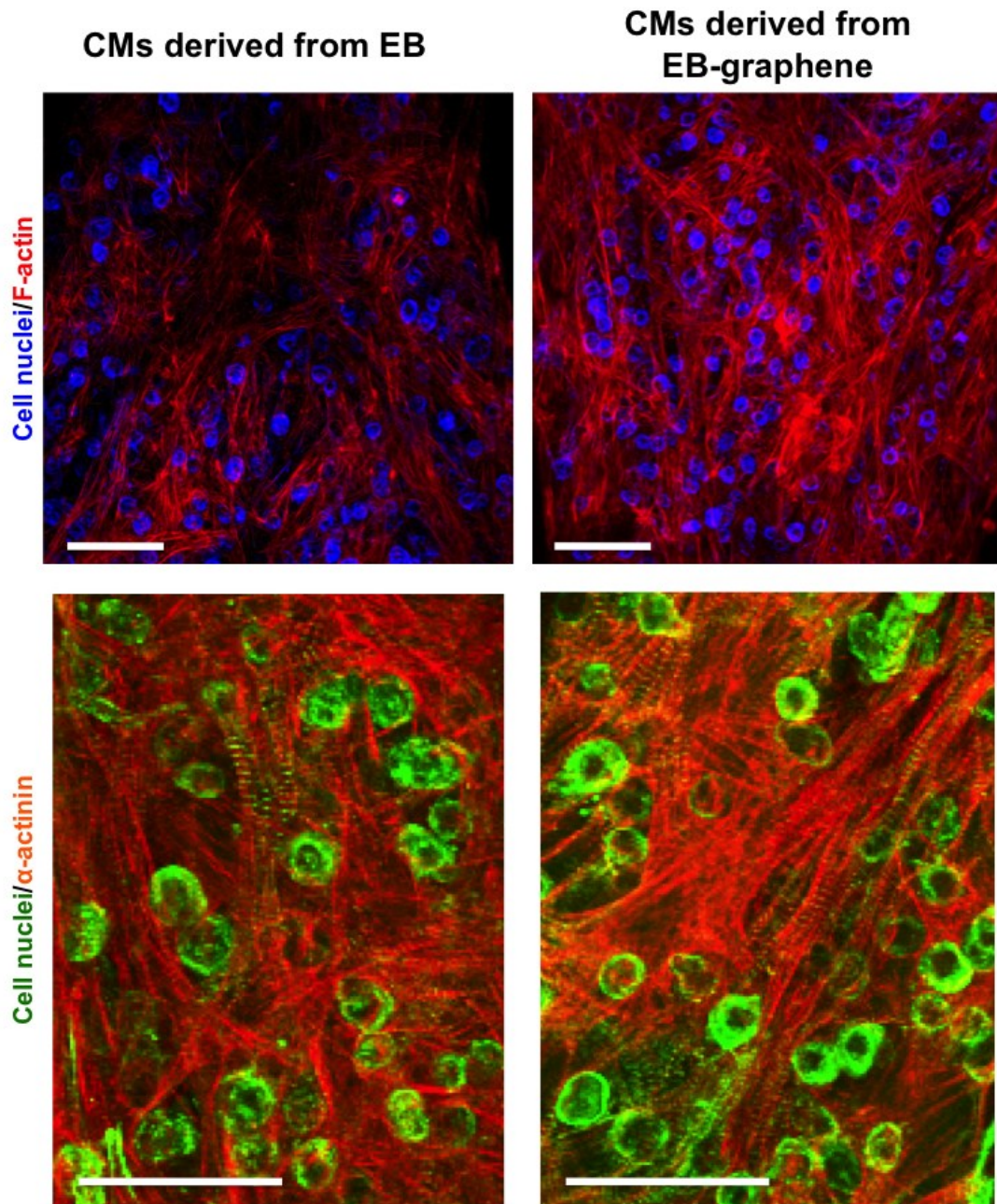


Figure S3. Immunostaining of F-actin (red) and cell nuclei (blue) for cardiomyocytes derived from EB and EB-graphene (Top pictures). Down pictures show immunostaining of sarcomeric α -actinin (red) and cell nuclei (green) for cardiomyocytes derived from EB and EB-graphene. The sarcomeric bands were visible in the down pictures. The scale bars show 50 μm .

Table S1. Genes and their primer sequences.

Gene type	Gene symbol	Left primer (5'→3')	Right primer (3'→5')
Housekeeping	GAPDH	tgtccgtcgtggatctgac	cctgcttcaccaccttcttg
	Actb	aaggccaaccgtgaaaagat	gtggtacgaccagaggcatac
	Ldha	ggcactgacgcagacaag	agcttgatcacctcgtaggc
	Tbp	ccaatgactcctatgacccta	cagccaagattcacggtagat
	Pgk1	aatctctgctgggcaagga	gaaagcggagggtttccag
Pluripotency	Nanog	ttcttgcttacaagggtctgc	agaggaaggcgaggaga
	Pou5f1	tgggcgttctctttggaa	gttgtcggcttctccac
	Dnmt3b	aatccagggccttcttcag	tggcaccctcttcttcattc
	Sox2	acggcagctacagcatga	gacgtcgtacgggtgcat
	Afp	gttctggcatgctgcaaag	ggatgctctctttgtctggaa
Endoderm	Sox17	cacaacgcagagctaagcaa	cgttctctgccaaggtc
	Gsc	ggagacgaagtaccagacg	cggcggttcttaaccag
	Foxa2	gagcagcaacatcaccacag	cgtaggccttgaggccat
	Pax9	gcagtgaatggattggagaag	cacagctgggagaccattc
	Gata6	ggtctctacagcaagatgaatgg	tggcacaggacagtccaag
	Hnf4a	cagcaatggacagatgtgtga	tggatgatggctgtggagtc
	Sox7	cacgctgcctgagaaaaac	gggagtactcaccctgtcc
	Tat	tgggaattcacagagcggta	acccggaagaaatttgggta
	Nes	ctgcaggccactgaaaagtt	tctgactctgtagaccctgcttc
	Pax6	ccgcacttagtcaacaaatgg	gtgagagcaattctcagatcctt
Ectoderm	Chat	ggttcggtgcgtaacagc	gcgattcttaatccagagtagca
	Tubb3	gcgcatcagcgtatactacaa	catggttccagggtccaagt
	Dlg4	gtgacgacccatccatcttt	ccggacatccacttcattg
	Fgf5	aaaacctgggtgcaccctaga	catcacattcccgaattaagc
	Syn1	cccagatgggtcgactacaca	ccacagggtatgttgctg
	Ncam1	cagctcactttgtgttcagga	acaatgaggatgcccacaat
	Gfap	acagactttctccaacctccag	ccttctgacacggatttgggt
	Pax2	gaggaaacgcgaggaaga	actcgatccagagcttcag
	T	actggcttagcctcgagtg	ccattgctcacagaccagag
	Mesp1	acccatcggttctgtacgc	gcatgtcgtgtgaagag
Mesoderm	Wnt3a	ggagaagagctatgaacttctga	gccagcagattccatacca
	Wnt8a	cagttttcaaccacaacagg	ctgcagttcttgggtactgc
	Mixl1	ccatgtaccagacatccact	cggttctggaaccacacct

Cardiac muscle	Nkx2-5	gacgtagcctggtgtctcg	gtgtggaatccgtcgaaagt
	Myh7	ctggagaaagagaaaagcgagt	aggtccggcacatcttctc
	Myh6	cgcatcaaggagctcacc	cctgcagccgcattaagt
	Myl2	ggacacatttgctgcccta	atcgtgaggaacacggtga
	Actc1	ggtcatcaccattggcaac	atgccagcagattccatacc
	Tnnt2	tggagggtagatccagaagc	tcctctctgccaggatcttc
	Isl1	gcaacccaacgacaaaactaa	ccatcatgtctctccggact
	Gata4	ccccctattaagcctcagc	caccctcggcattacgac
	Tbx5	aattgagaacaaccccttcg	cctgggaaccacaggatactc
	Myl7	gaaggagacctattcccagctc	gagtgtgaggaagacggtgaa
	Mef2c	gtcagcacactgggaaacc	agatctccgcccatcaga
	Kdr	ccccaaattccattatgacaa	cggctctttcgcttactgtt
	Pecam1	gctggtgctctatgcaagc	atggatgctgttgatggtga
	Tek	ggtgctactgagcaacttagtcc	ctggttgaggaggagagaatg
	Pdgfrb	tgatgaaggctctccagagg	ctgcttgctgtggctcttct
Blood vessel	Cdh5	tcatcaaaccacgaagtcc	ggtctgtggcctcaatgtaga
	Flt1	aagactcgggcacctatgc	ggttttgaagcaggtgtgg
	Etv2	cattgactcgtactccaaaact	tggaaactcgggctattg
	Flii	gtctgaaggcttgccaggta	cattacctttgcggaccag
	Gata2	cactctggacacatcctaccc	tagcccatggcagtcacc
	Runx1	ctccgtgctaccactcaact	atgacggtgaccagagtgc
	Klf1	ggcgaactttggcaccta	caggcataaggcttctctcc
	Gata1	cgagaccatcgatctttgtg	gggaacactggggttgaa
	Hbb-bh1	tggatcctgagaacttcaagc	cattggccactccaatcac
	Rb1	tgcaaatacagagacacaagca	cggagatatgctagacggtaca
Skeletal muscle	Sox6	gctgggtgatcctcgtgta	tcccttgaggtaagtctctg
	Tagln	ggcctttaaacccctcacc	gccatgttgaggcagagaag
	Myog	ctacaggccttgctcagctc	cgtgtgtggagttgcatt
	Actg2	catcaccaactgggatgaca	tgtttgctttggggttagg
	Vim	cctgcagtcattcagacagg	cgtgcagtttctcaaaaagg
	Acta2	ccaaccgggagaaaatgac	cagttgtacgtccagaggcata

Table S2. Significantly expressed genes in the differentiation of the EB-graphene in contrast with the EB at day 5 of culture. The gene expression levels were normalized with respect to the internal reference gene GAPDH.

Gene type	Gene symbol	Average expression in the EB	Average expression in the EB-graphene	p-value
Endoderm	Foxa2	0.92±0.13	1.14±0.14	0.013
Ectoderm	Ncam1	1.20±0.48	2.02±0.66	0.026
Cardiac muscle	Isl1	0.85±0.23	1.43±0.41	0.009
	Tbx5	0.50±0.23	1.07±0.63	0.049
	Mef2c	0.58±0.20	1.04±0.48	0.047
Blood vessel	Pecam1	0.88±0.17	0.68±0.09	0.021
Hematocyte	Runx1	1.01±0.33	0.61±0.24	0.030
Skeletal muscle	Vim	0.94±0.10	0.82±0.06	0.031

Table S3. Significantly expressed genes in the differentiation of the EB-graphene in contrast with the EB at day 10 of culture. The gene expression levels were normalized with respect to the internal reference gene GAPDH.

Gene type	Gene symbol	Average expression in the EB	Average expression in the EB- graphene	p-value
Pluripotency	Nanog	3.51±2.67	1.04±0.46	0.043
Endoderm	Afp	1053.99±702.12	374.39±203.15	0.040
Ectoderm	Dlg4	2.23±0.41	3.30±0.59	0.004
Cardiac muscle	Myh6	708.31±380.24	1392.94±496.75	0.022
	Actc1	1129.16±543.80	2269.40±651.90	0.007
	Tnnt2	559.03±300.85	2055.26±1118.02	0.010
	Myl7	364.08±113.36	595.86±131.36	0.007
	Mef2c	3.26±1.38	6.26±1.33	0.002
Blood vessel	Kdr	0.57±0.12	0.78±0.17	0.030
	Tek	11.22±2.58	17.22±2.97	0.003
	Cdh5	10.92±3.44	15.25±3.18	0.045
	Etv2	0.14±0.08	0.31±0.08	0.004
Hematocyte	Sox6	7.22±1.46	12.83±2.12	0.002
Skeletal muscle	Vim	2.14±0.34	2.95±0.71	0.031
	Acta2	308.67±166.43	600.81±132.32	0.006

Table S4. Significantly expressed genes during the differentiation of the EB-graphene in contrast with the EBs at day 15 of culture. The gene expression levels were normalized with respect to the internal reference gene GAPDH.

Gene type	Gene symbol	Average expression in the EB	Average expression in the EB- graphene	p-value
Endoderm	Foxa2	1.06±0.23	0.64±0.26	0.019
	Gata6	1.07±0.34	0.58±0.19	0.014
	Hnf4a	3.98±0.89	1.56±0.89	0.001
	Sox7	1.72±1.00	0.50±0.23	0.019
	Tat	4.29±3.27	0.59±1.00	0.029
Ectoderm	Dlg4	2.43±0.47	1.81±0.40	0.038
	Pax2	61.39±43.54	3.20±3.18	0.001
Blood vessel	Tek	3.60±0.81	2.18±1.25	0.050
	Cdh5	5.14±1.67	2.17±1.47	0.010
	Gata2	1.69±0.89	0.54±0.25	0.015