

## Supplementary Materials for **Graphene biointerfaces for optical stimulation of cells**

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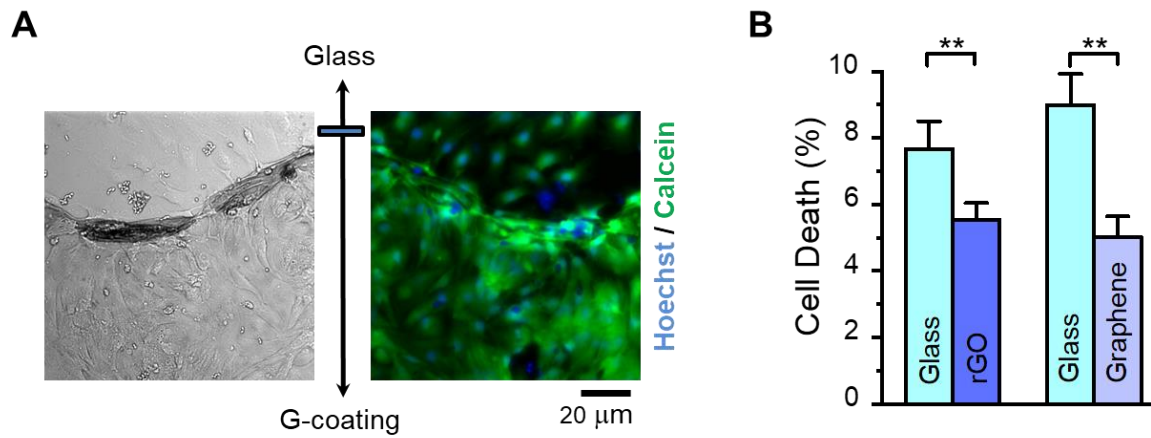
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### The PDF file includes:

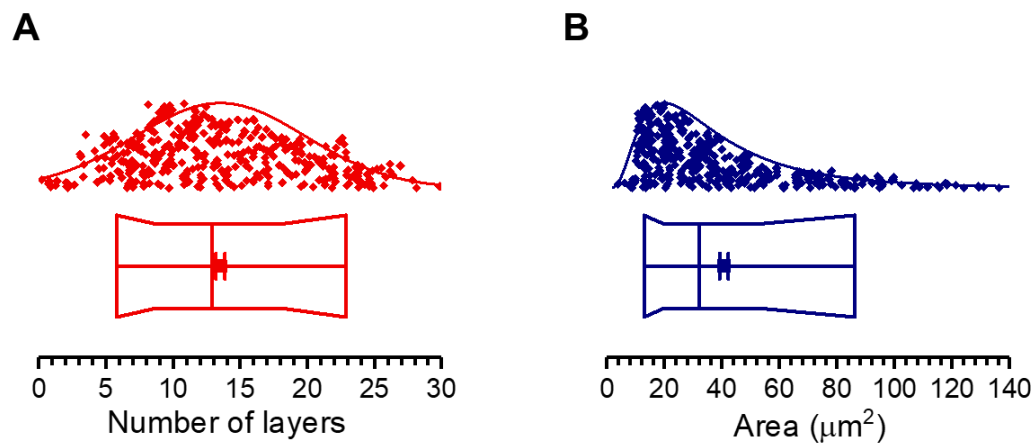
- fig. S1. Biocompatibility of G-biointerfaces.
- fig. S2. Geometrical characteristics of G-flakes.
- fig. S3. Basal heart rates in control and G-treated zebrafish ( $n = 7$  for each group) 2 hours after injection.
- Legends for movies S1 to S4

### Other Supplementary Material for this manuscript includes the following: (available at [advances.sciencemag.org/cgi/content/full/4/5/eaat0351/DC1](https://advances.sciencemag.org/cgi/content/full/4/5/eaat0351/DC1))

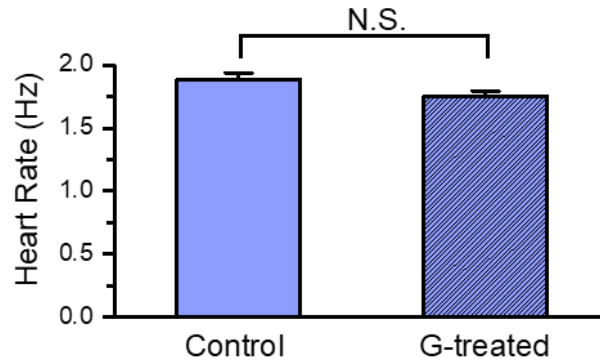
- movie S1 (.avi format). Optical stimulation of contractile activity in mouse embryonic stem cell–derived CMs cultured on rGO-coated coverslips (green light, 4.6 mW/mm<sup>2</sup>).
- movie S2 (.avi format). Optical stimulation of contractile activity in mouse embryonic stem cell–derived CMs via rGO flakes using the same conditions as in movie S1.
- movie S3 (.avi format). Effects of light illumination on the heart contractions in a 3-dpf zebrafish embryo injected with G-biointerfaces (0.5 mg/ml) dispersed in PBS.
- movie S4 (.avi format). Automated image analysis of CM contractions.



**fig. S1. Biocompatibility of G-biointerfaces.** (A) Representative bright-field (left) and fluorescent (right) light microscopy images of neonatal rat ventricular cardiomyocytes cultured on a heterogeneous substrate as marked (the upper part is the glass, and the lower part is the rGO-coated surface). (B) Summary of cell viability experiments for cardiomyocyte cultures on control, rGO- and graphene-coated coverslips. Data are presented as mean  $\pm$  SEM. ( $n \geq 100$  cells per each condition). \*\*\*,  $P < 0.005$ , unpaired  $t$ -test.



**fig. S2. Geometrical characteristics of G-flakes.** Data and Box Plots of the area (A) of rGO flakes and the number of layers in rGO flakes (B). The box shows 10%, 25%, 75%, 90% of the data, the horizontal bar is the median, the closed symbol is the mean, and the whiskers are the SEM ( $n = 375$ ).



**fig. S3. Basal heart rates in control and G-treated zebrafish ( $n = 7$  for each group) 2 hours after injection.**

### CAPTIONS FOR MOVIES

**movie S1. Optical stimulation of contractile activity in mouse embryonic stem cell–derived CMs cultured on rGO-coated coverslips (green light,  $4.6 \text{ mW/mm}^2$ ).** Green circle appears in the top left corner of a movie during an optical stimulation event. Note that mCherry fusion protein expressed in these cardiomyocytes is also excited by green light. Therefore, mCherry fluorescent signal can be used as an indicator of an optical stimulation event. Scale bar:  $50 \mu\text{m}$ .

**movie S2. Optical stimulation of contractile activity in mouse embryonic stem cell–derived CMs via rGO flakes using the same conditions as in movie S1.** Scale bar:  $20 \mu\text{m}$ .

**movie S3. Effects of light illumination on the heart contractions in a 3-dpf zebrafish embryo injected with G-biointerfaces ( $0.5 \text{ mg/ml}$ ) dispersed in PBS.** During stimulation with green light ( $0.8 \text{ mW/mm}^2$ ), a filled circle appears in the top left corner of the video. Scale bar:  $100 \mu\text{m}$ .

**movie S4. Automated image analysis of CM contractions.** The top part of the video shows an example of bright-field imaging of cardiomyocyte contractions. Green crosses indicate the center

of a subset of regions of interest (ROIs) that were selected for displacement analysis based on their maximum intensity variance. Red crosses indicate the center of dynamic positions of these ROIs in each time point that were calculated using the maximum correlation analysis. Scale bar: 10  $\mu\text{m}$ .

The bottom part of the video shows a temporal trace of mean displacement values for ROIs in the top part of the video. Displacement values were calculated as a distance traveled by ROIs. Optical stimulation event is indicated by the green circle in the left top corner of the video and the green bar on the displacement trace.