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Supplementary Information

Ultralarge suspended and perforated graphene membranes for cell culture applications

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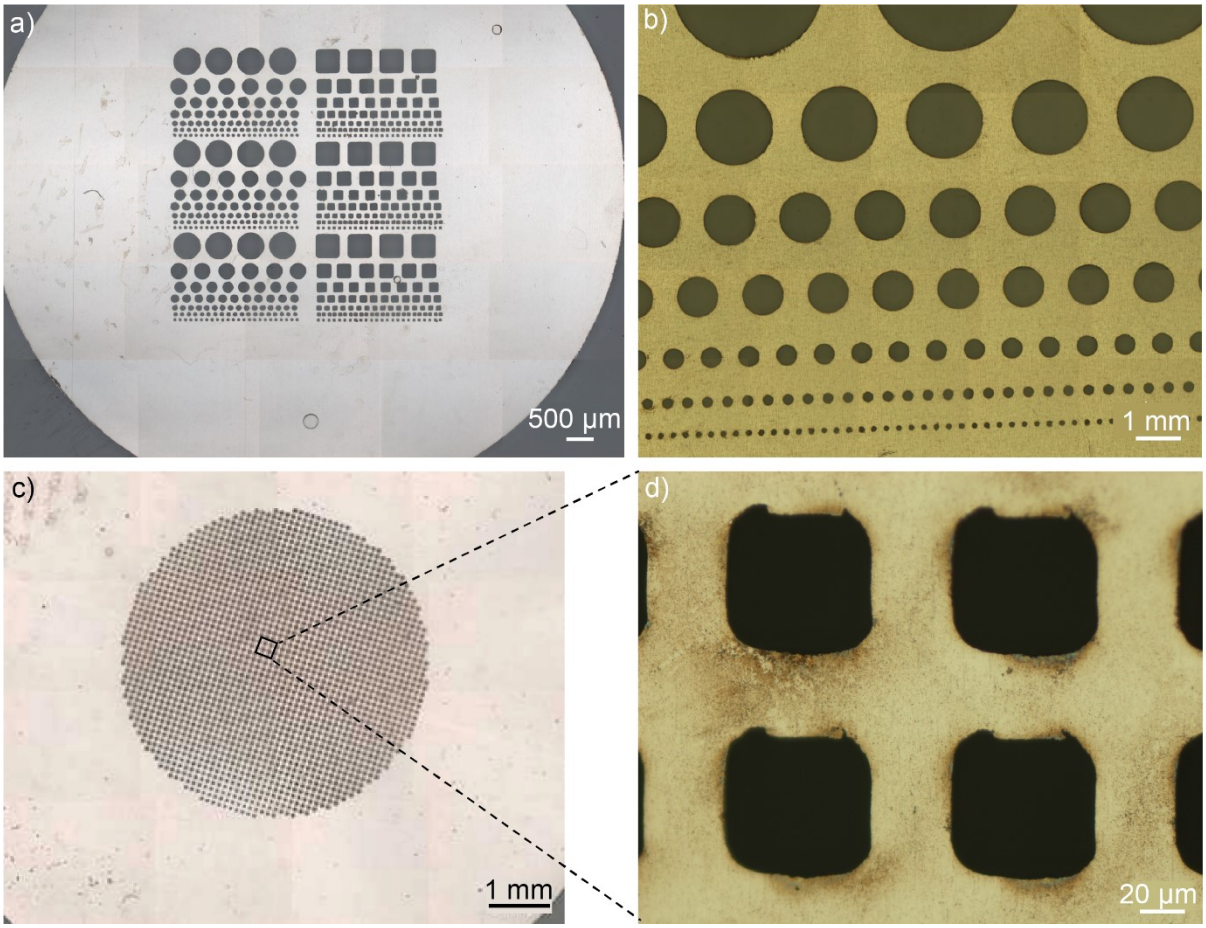

Figure S1: Supportive grid designs. a) Image of a grid with arrays of circular and square holes (diameters of 55 to 500 µm). b) Image of a substrate with circular perforation diameter between 100 µm and 3 mm. c,d) Image of a grid composed of ~2000 square holes (60 µm x 60 µm; 40 µm bridge).

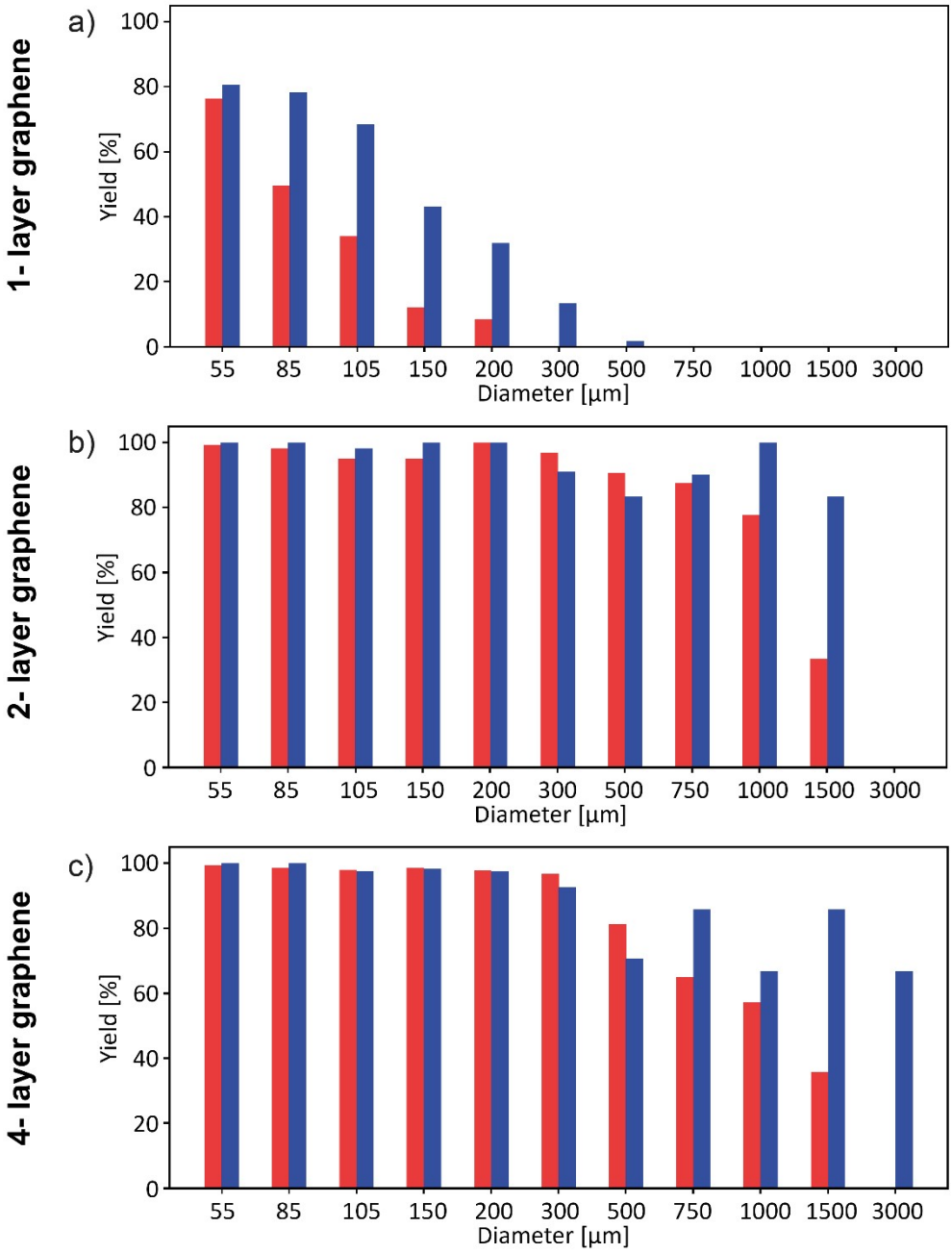

Figure S2: Transfer of graphene membranes using the anthracene sublimation method. Yield of membranes for single-layer (a), bi-layer (b), and 4-layer (c) of commercial (Graphenea) graphene (blue) or in-house graphene (red). Considerable differences in transfer yield were observed for single layer graphene in dependence of the graphene material used, which might be due to differences in the size of grain boundaries, the graphene coverage or the smoothness of the growth substrate during the CVD process.

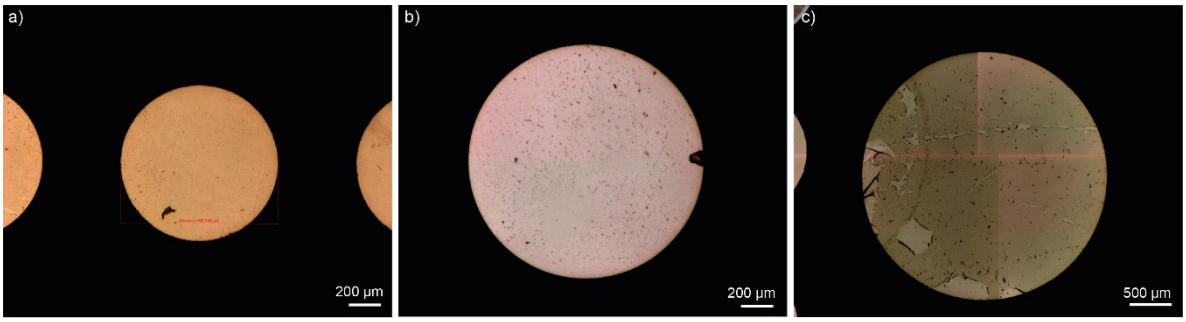

Figure S3: Millimeter sized suspended graphene. Optical microscope images of bilayer graphene suspended over 1 mm (a), 1.5 mm (b) as well as 4-layers graphene suspended over 3 mm (c).

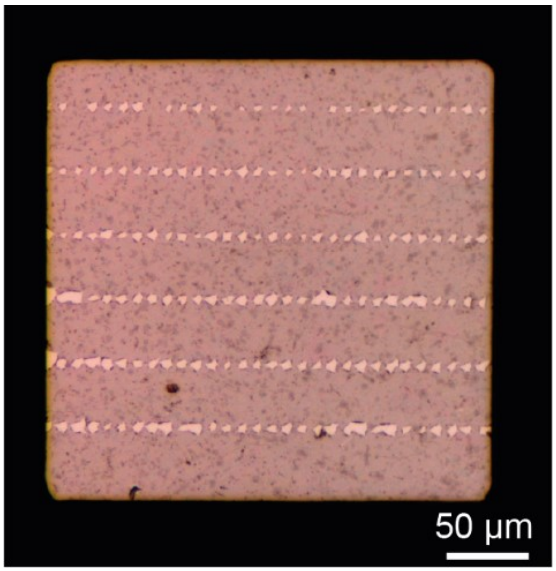

Figure S4: Perforation of millimeter sized suspended graphene. Optical microscope image of perforated bilayer graphene suspended over a 0.5 mm hole of the supportive grid. The laser intensity was decreased for each "perforation line" starting from the bottom of the square shaped suspended graphene (1.75×10^{-8}, 1.64×10^{-8}, 1.54×10^{-8}, 1.47×10^{-8}, 1.37×10^{-8},1.30×10^{-8} J). Perforated pores were up to 10 µm diameter.

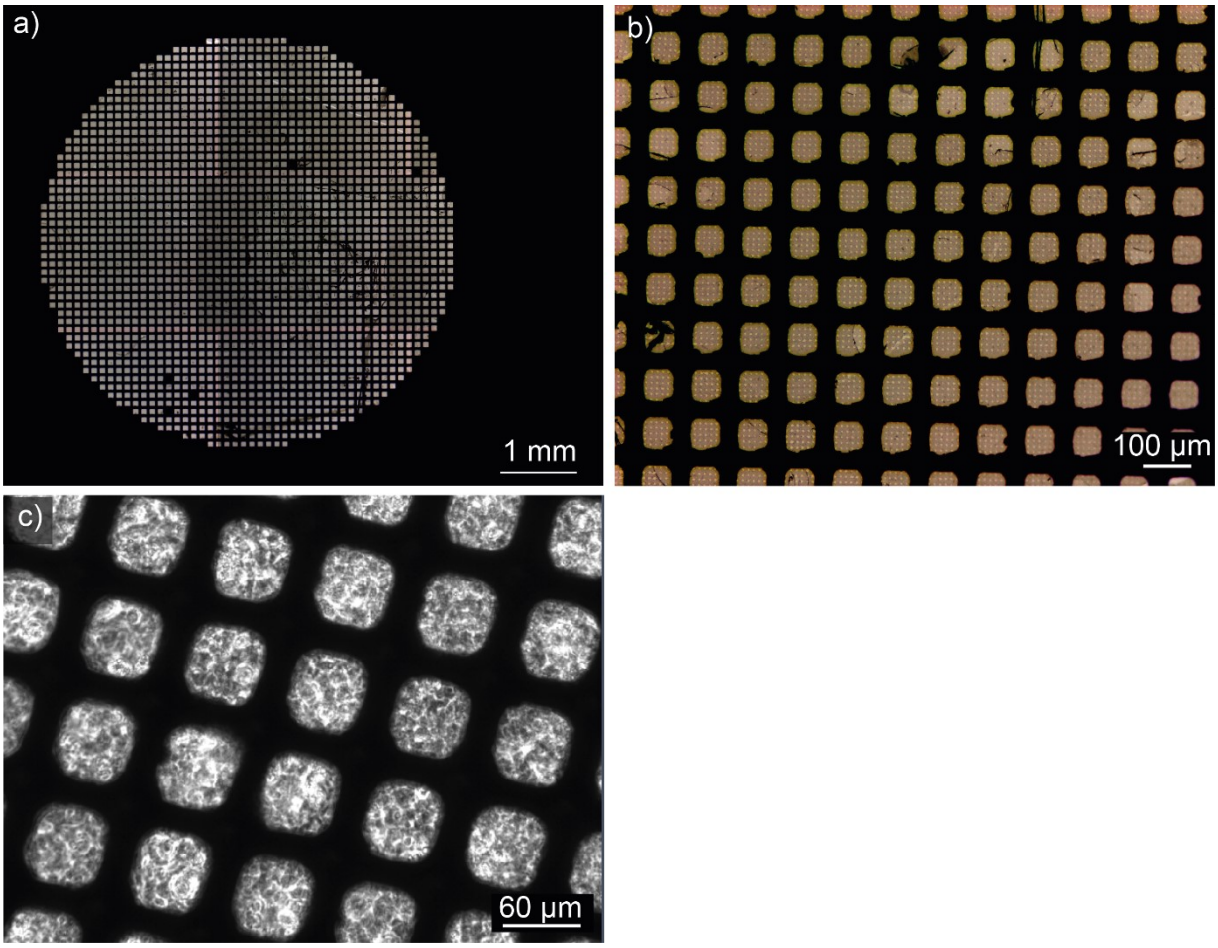

Figure S5: Suspended graphene for cell cultivation. a) Optical microscopy image of a grid (60 µm x 60 µm; 40 µm bridge) covered with 2-layer suspended graphene. b) Optical microscopy image of suspended graphene after pulsed laser ablation. c) Optical microscopy image of BeWo cells cultivated on suspended graphene membranes.

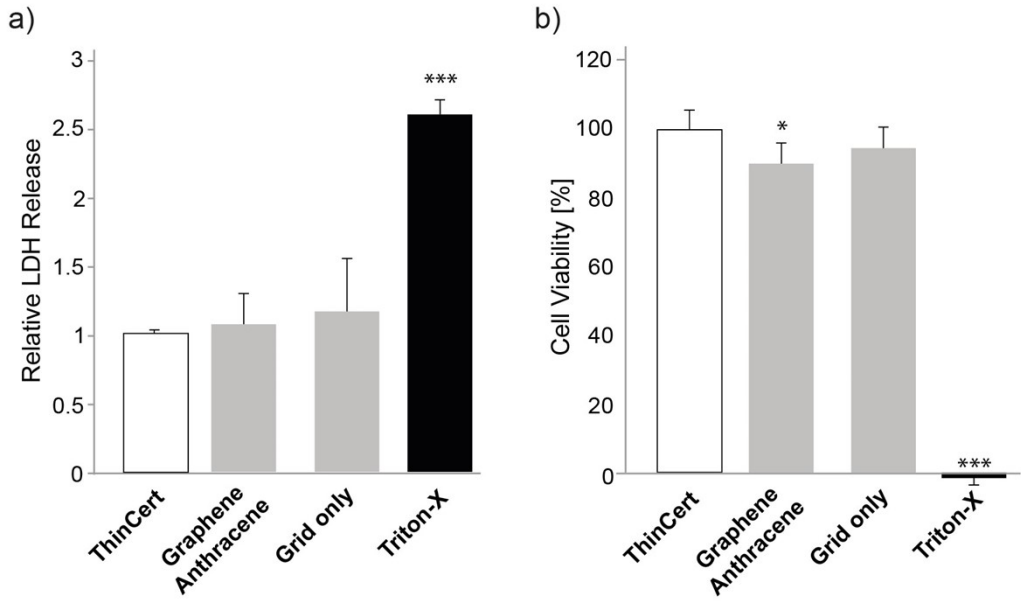

Figure S6: Viability of BeWo cells on suspended graphene membranes. a) LDH assay conducted after 24 h of cell growth on the different membranes to assess cytotoxicity. b) MTS assay conducted after 24 h of cell growth to assess the cell viability/metabolic activity of the cells cultured on the different membranes. Results represent mean ± STD of 3 independent experiments with 3 technical replicates each. * = $p < 0.5$; ** = $p < 0.1$; *** = $p < 0.01$.

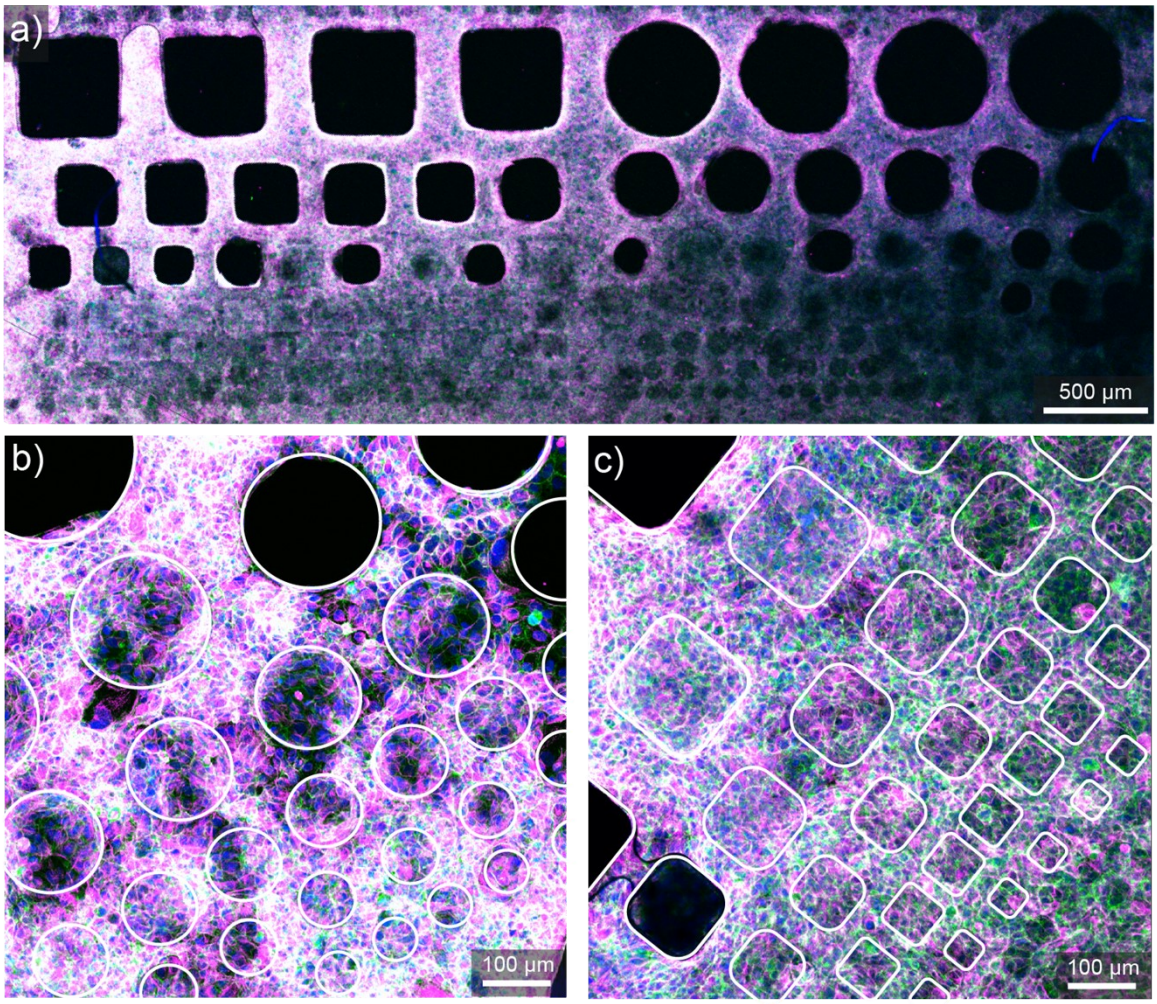

Figure S7: Cell cultivation on micro- to millimeter-sized suspended perforated graphene membranes. BeWo cells were cultivated on a perforated bilayer graphene membrane mounted on gradient grids with increasing size of the openings to identify the maximum permissive size for cell cultivation without membrane rupturing. a) Overview of gradient grids showing the BeWo cells cultivated on suspended, perforated graphene membranes after immunocytochemical staining for tubulin (red), adherens junctions (γ-catenin; green), and cell nuclei (DAPI; blue). Black openings indicate ruptured membranes. b and c) Close-up images of cells on top of perforated graphene suspended on circular (b) or square (c) openings of increasing sizes.