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Establishment of pathogen-free human pluripotent stem cell culture method using graphene that meets clinical-grade

The maintenance of undifferentiated human pluripotent stem cells (hPSC) under xeno-free condition requires the use of human feeder cells or extracellular matrix (ECM) coating. However, human-derived sources may cause human pathogen contamination by viral or non-viral agents to the patients. Here we demonstrate feeder-free and xeno-free culture system for hPSC expansion using diffusion assisted synthesis-grown nanocrystalline graphene (DAS-NG), a synthetic non-biological nanomaterial which completely rule out the concern of human pathogen contamination. DAS-NG exhibited advanced biocompatibilities including surface nanoroughness, oxygen containing functional groups and hydrophilicity. hPSC cultured on DAS-NG could maintain pluripotency in vitro and in vivo, and especially cell adhesion-related gene expression profile was comparable to those of cultured on feeders, while hPSC cultured without DAS-NG differentiated spontaneously with high expression of somatic cell-enriched adhesion genes. This feeder-free and xeno-free culture method using DAS-NG will facilitate the generation of clinical-grade hPSC.

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References

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[2] Kwak J., *et al*., Nature commun, 3 (2012) 645
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Figures

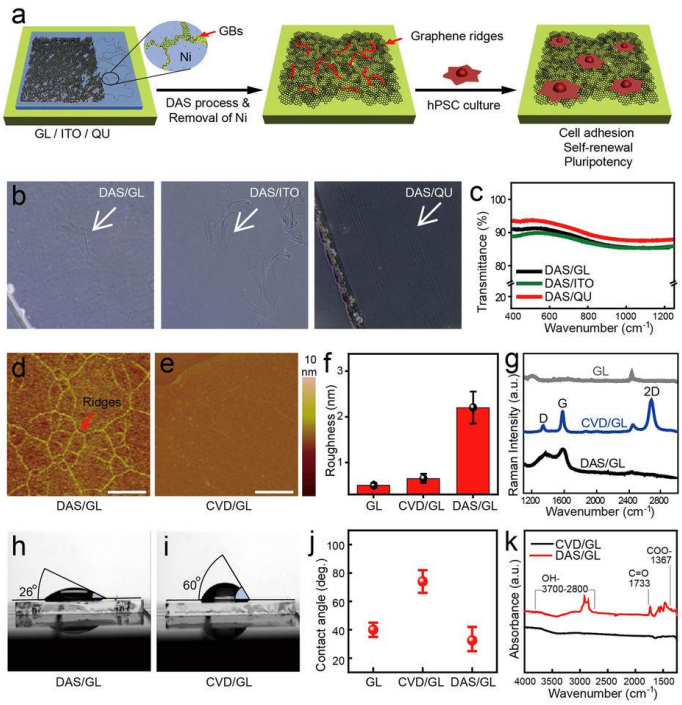

Figure 1: Structural and optical properties of DAS-NG coated culture substrates. (a) Schematic diagrams of diffusion assisted synthesis-grown nanocrystalline graphene (DAS-NG) preparation on transparent substrates including GL, ITO, and QU for hPSC cultivation. (GBs, Grain boundaries; Ni, Nickel). (b) Optical microscopy images of DAS-NG layers grown at 260 °C on GL, ITO and QU. Graphene layers are indicated with white arrows. (c) Transmittances of DAS/GL (black), DAS/ITO (green) and DAS/QU (red). (d,e) AFM images of (d) 3 dimensional DAS-NG layers on GL with high-density multilayer graphene ridges (red arrow) and (e) 2 dimensional CVD graphene layers on GL. (f) Plot of surface Root-mean-square roughness from AFM images (5 × 5 μm2) of GL, CVD/GL and DAS/GL. (g) Raman spectra of GL (grey), CVD/GL (blue) and DAS/GL (black). (h,i) Images of water drop (40 μl on 1.5 × 1.5 cm2) contact angle on (h) DAS/GL and (i) CVD/GL. (j) Plot of water contact angle measurements on bare GL, CVD/GL and DAS/GL. (k) FT-IR spectra of CVD/GL and DAS/GL. Scale bar, 1 μm (d,e). Data are presented as mean ± s.e.m (n = 3) (f,j).

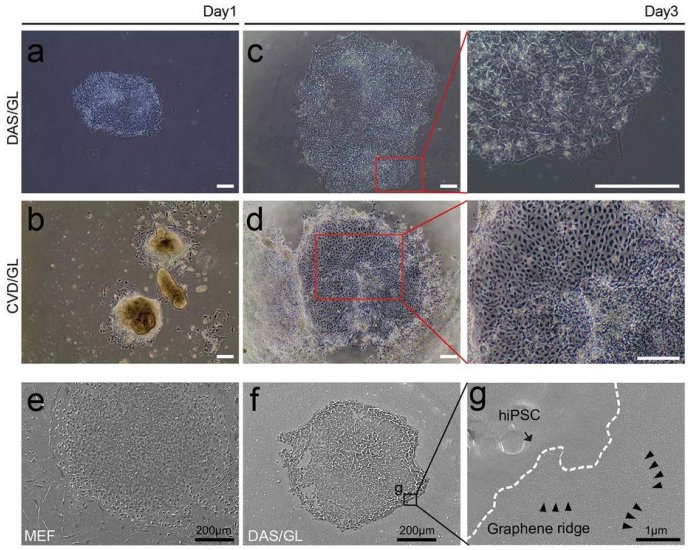

Figure 1: Feeder-free cultivation of hPSC on DAS-NG. (a,b) Morphology of hiPSC seeded on (a) DAS/GL and (b) CVD/GL at day 1. (c,d) High magnification of hiPSC grown on (c) DAS/GL and (d) CVD/GL at day 3. (e–g) SEM images of hiPSC cultured on (e) MEF, (f) DAS/GL at day 3 and (g) Zoomed inset shows hiPSC (arrow) attached on graphene ridges (arrow heads) of DAS-NG. (h–j) Morphology of hiPSC colonies cultured on (h) DAS/GL, (i) MEF and (j) bare GL at 2 weeks.