Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B. This journal is © The Royal Society of Chemistry 2020

Electronic Supplementary Information

2

1

3 Ultra-thin, transparent, porous substrates as 3D culture

4 scaffolds for engineering ASC spheroids for high-

5 magnification imaging

- 6 Yoshitaka Suematsu¹, Ya An Tsai¹, Shinji Takeoka¹, Clemens M. Franz², Satoshi Arai²,
- 7 Toshinori Fujie^{3,4}*
- 8 1. Graduate School of Advanced Science and Engineering, Waseda University, TWIns,
- 9 2-2, Shinjuku-ku, Tokyo 162-8480, Japan
- 10 2. WPI Nano Life Science Institute, Kanazawa University, Kakuma-machi, Kanazawa
- 11 920-1192, Japan
- 12 3. School of Life Science and Technology, Tokyo Institute of Technology, B-50, 4259
- 13 Nagatsuta-cho, Midori-ku, Yokohama 226-8501, Japan
- 14 4. Research Organization for Nano & Life Innovation, Waseda University, 513
- 15 Wasedatsurumaki-cho, Shinjuku-ku, Tokyo 162-0041, Japan
- 16 * Corresponding author Toshinori Fujie (t_fujie@bio.titech.ac.jp)

1 Supporting Figure



Figure S1. (a) SEM image of an adherent spheroid on the porous nanosheet (the arrow
indicates the extended cellular filopodia, scale bar: 10 μm). (b) Phase contrast image of
an adherent spheroid on the porous nanosheet (the arrows indicate the extended cellular
filopodia, scale bar: 50 μm).





8

- 9 Figure S2. 3D model of a spheroid formed on the porous nanosheet reconstructed from
- 10 the fluorescent images of Figure 6d by using ImageJ.
- 11



2 Figure S3. Quantification of secreted FGF-2 from spheroids and monolayer cells. The

3 data represent the average (n = 2).

4

1



- 6 Figure S4. Fluorescence and phase contrast images of immunostained collagen IV
- 7 secreted from ASCs cultured on the (a) porous nanosheet and (b) porous nanosheet (FN)
- 8 (scale bars: $20 \ \mu m$).
- 9

10 Supporting Movies

- 11 Movie S1: The morphology of ASCs migrating on the porous nanosheet captured with
- 12 $40 \times$ magnification every 30 s for 2 h.

- 1 Movie S2: The morphology of ASCs migrating on the flat nanosheet captured with $40 \times$
- 2 magnification every 30 s for 2 h.
- 3 Movie S3: The morphology of ASCs migrating on NanoCulture Dish captured with $10 \times$
- 4 magnification every 60 s for 6 h.
- 5 Movie S4: The morphology of ASCs migrating on the porous nanosheet captured with
- 6 $10 \times$ magnification every 60 s for 6 h.