Supplementary information

In situ paper-based 3D cell culture for rapid screening the anti-melanogenic activity

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Optimization of cell number and test zone diameter

To determine the optimal cell number and test zone diameter for melanoma cell culture, Whatman No.1 and Whatman No.4 were used for culturing B16F10 cells in various concentrations as follows: 100,000, 200,000, 400,000, and 800,000 cells/test zone in designed paper with 5 mm and 10 mm test zone diameters. Figure S1 shows that the intensity of melanin has a direct variation with the cell number in both Whatman No.1 and Whatman No.4, except Whatman No.4 with 10 mm diameter test zone, which shows low melanin intensity at 200,000 cell concentration due to the excessive test zone area causing an inconsistent distribution of melanin on the paper.

Supporting Figures

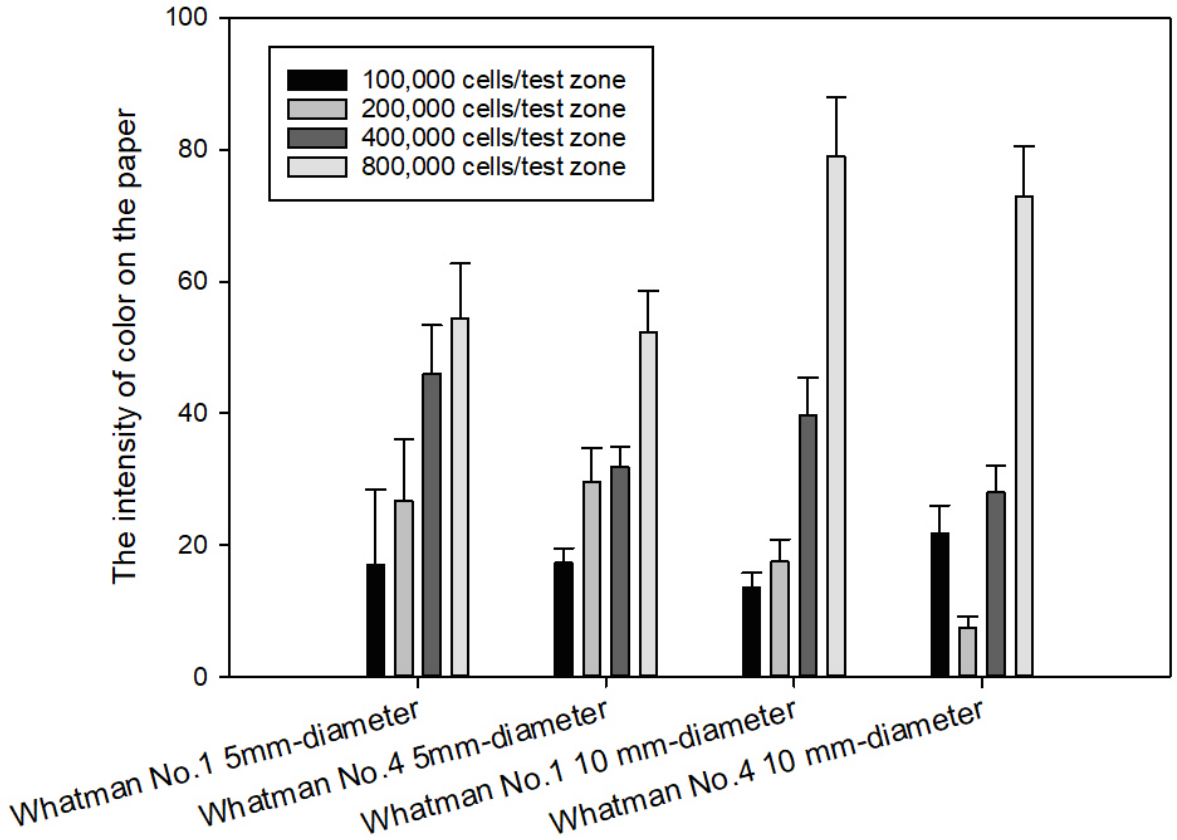
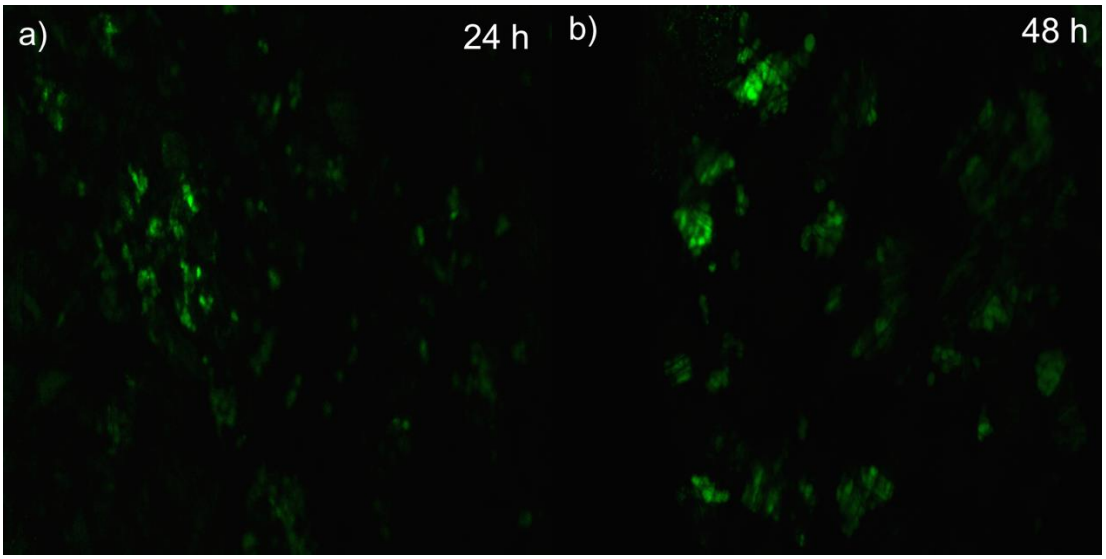


Figure S1. Optimization of cell number and test zone diameter. Data is presented as the mean ± SD (n=3).

Cell viability assay of melanoma cells on the paper

To measure cell viability, B16F10 cells were cultured on paper for 24, 48, 72, 96, and 144 hours without changing the culture medium. After that, viable cells were stained with calcein-AM and dead cells were stained with propidium iodide (PI). Figure S2 shows that cells were able to proliferate under the paper-based cell culture. The result shows single viable cells in the paper after 24 hours of culturing. After 48 hours of culturing, cells started proliferating and forming clusters of cells. After 72 and 96 hours, cell numbers were higher than after 24 and 48 hours of incubation with some dead cells shown in red color. After 120 hours, the cells continued forming clusters, covering almost the entire area of paper thus indicating cell proliferation.



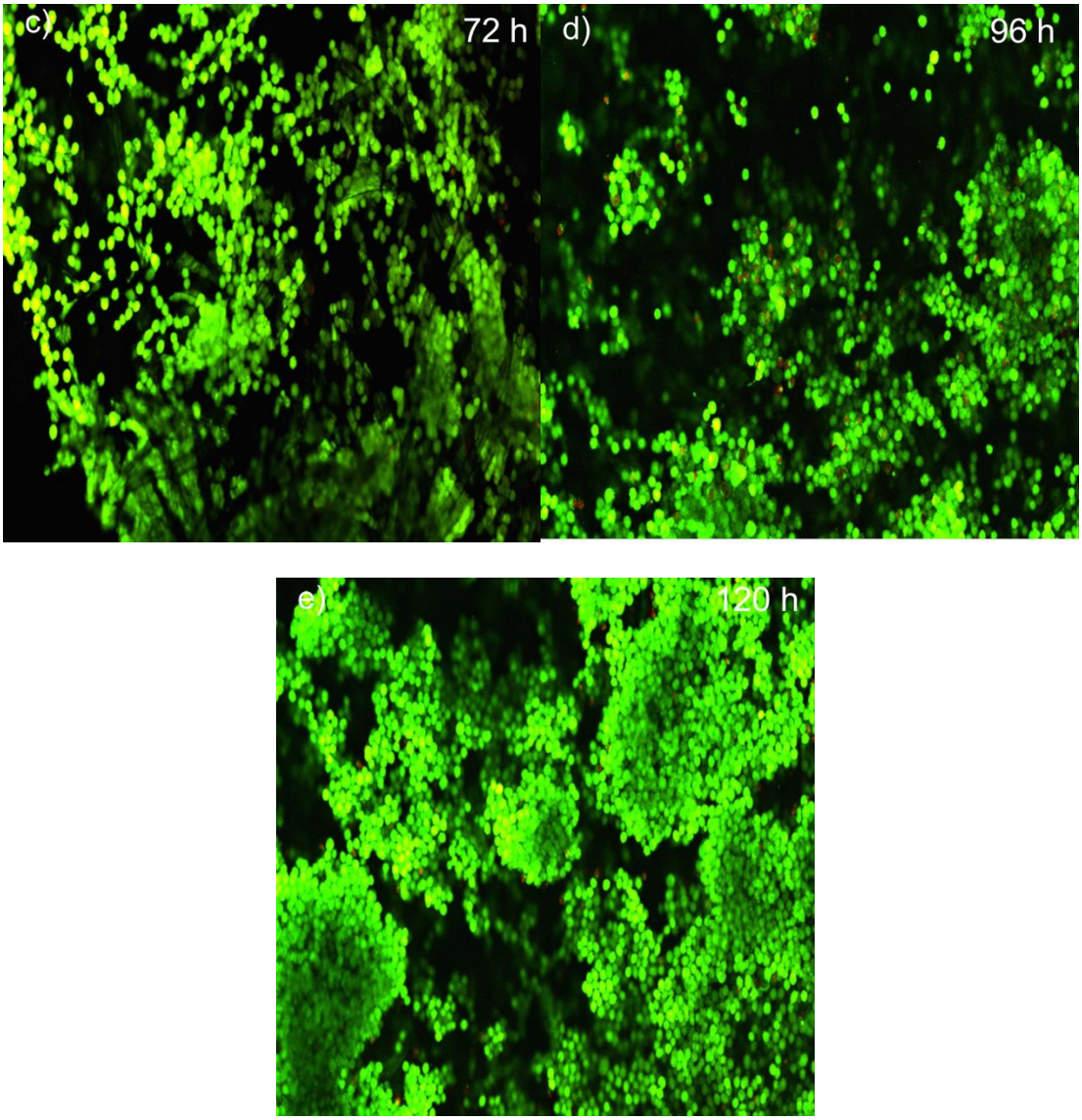


Figure S2. Fluorescent images of B16F10 cells after culturing for a) 24, b) 48, c) 72, d) 96, and e) 120 hours (Green – GAPDH; Red – Propidium iodide; 100-micron resolution images)