Electronic Supplementary Material (ESI) for Soft Matter.

Electronic Supplementary Information

A. A simplified surface energy model

We consider the following three morphologies, as shown in Figure S5a. Assuming the mixed spheroids are concentric spheres in shape, then the total surface energy of the three different morphologies can be written as

$$E_1 = 4\pi * (r_g^2 \sigma_{rg} + R^2 \sigma_r) \quad (1)$$

$$E_2 = 4\pi * (r_r^2 \sigma_{rg} + R^2 \sigma_g) \quad (2)$$

$$E_3 = 4\pi * \left(r_g^2 \sigma_g + r_r^2 \sigma_r\right), \quad (3)$$

where r_g is the radius of the green (MCF10A) cell aggregate, r_r is the radius of the red (MDA-MB-231) cell aggregate, and R is the radius of the spheroid, σ_{rg} is the interfacial tension between the two types of cells, σ_r is the surface tension of the red aggregate (in culture medium) and σ_g is the surface tension of the green aggregate [Note that $\sigma_g > \sigma_r$ since the green MCF10A cells are known to be more cohesive (or has more adhesion molecules E-cadherin) than the red MDA-MB-231 cells](Figure S5b).

A.1. Core-shell reversal

The core-shell morphology reversal observed in Figure 3 can be explained as a result of differential cell growth rate of MDA-MB-231 and MCF10A cells using the simplified surface energy model. Using the above equations (1) & (2), we obtain the surface energy difference of the spheroids between Morphology 1 & 2 to be:

$$\frac{E_1 - E_2}{4\pi\sigma_{rg}R^2} = \left[\left(\frac{N_g}{N}\right)^{\frac{2}{3}} - \left(\frac{N_r}{N}\right)^{\frac{2}{3}}\right] - \frac{\sigma_g - \sigma_r}{\sigma_{rg}}, \quad (4)$$

where N_r and N_g are the respective numbers of red (MDA-MB-231) cells and green (MCF10A) cells in the mixed aggregate, and $N = N_g + N_r$. Here, we assume all the cells have the same size, thus $r_g/R = (N_g/N)^{1/3}$ and $r_r/R = (N_r/N)^{1/3}$.

Further simplifying equation (4), we have:

$$\frac{E_1 - E_2}{4\pi\sigma_{rg}R^2} = \left[\left(\frac{k}{1+k}\right)^{\frac{2}{3}} - \left(\frac{1}{1+k}\right)^{\frac{2}{3}}\right] - \beta \quad (5)$$

where $k = N_g/N_r$ and $\beta = (\sigma_g - \sigma_r)/\sigma_{rg}$. From Equation 5 and Figure S5c, d, we can see that Morphology 1 is always energetically more favorable (i.e. $E_1 - E_2 < 0$), when $\beta > 1$. However, if $0 < \beta < 1$, then which morphology is at a lower energy state depends on the cell number ratio N_g/N_r. Taking $\beta = 0.5$ as an example in Figure S5c, we see that $E_1 - E_2$ changes from negative to positive when the number ratio k passes a critical value of 4. In our experiments, green cells grow faster than red cells causing the number ratio to increase with time. This explains the core-shell morphology reversal shown in Figure 3. Given the fact that we observed the core-shell reversal, it suggests that the β value has to be within the range of $0 < \beta < 1$ for the untreated (naturally growing) MCF10A and MDA-MB-231 cells studied here. For the experiments where the cell growth was inhibited, no core-shell morphology reversal was observed. There might be two possibilities. One is that the growth-inhibition causes some unknown alteration on hetero-cohesion property and interfacial tension (σ_{rg}) between the two types of cells such that $\beta > 1$, and the Morphology 1 is always a stable state. The other possibility is that the cell proliferation plays some roles in cell self-organization that are absent in growth-inhibition condition. Future work is required to measure the interfacial tension, cohesion protein (e.g. cadherins) expression, cell motility and cell mechanical properties, which could all affect the self-organization.

A.2. From core-shell to side-by-side morphology

We next performed the same analysis for the energy difference of Morphology 1 and Morphology 3 as shown in Figure S5, and we have:

$$\boldsymbol{\frac{E_1 - E_3}{4\pi} = r_g^2(\sigma_{rg} - \sigma_g) + \sigma_r(R^2 - r_r^2).} \quad \textbf{(6)}$$

This can be simplified as:

$$\boldsymbol{\frac{E_1 - E_3}{4\pi\sigma_r N_r} = k^{\frac{2}{3}}\left(\frac{\alpha - 1 - \alpha\beta}{\beta}\right) + (1+k)^{\frac{2}{3}} - 1,} \quad \textbf{(7)}$$

where $\sigma_g = \alpha\sigma_r$.

To determine which energy state is more stable, it requires the knowledge of three variables here, α, β and k.

Since $k > 0$, for a large α so that $\alpha > 1/(1-\beta)$, $E_1 - E_3 > 0$, i.e. Morphology 3 (side-by-side) is always the more stable state.

The behavior of a smaller α ($1 < \alpha < 1/(1-\beta)$) requires numerical analysis. Given the above discussion in A.1., we consider a case when $\beta = 0.5$, Figure S6 shows the result of $E_1 - E_3$ as a function of α, β and k. Figure S6 shows that Morphology 1 is stable only when α is close to 1. In our experiments, this is not physical since the cohesion properties of green and red cells are very different, and therefore α should be much greater than 1 and Morphology 3 is a more stable state.

Figures

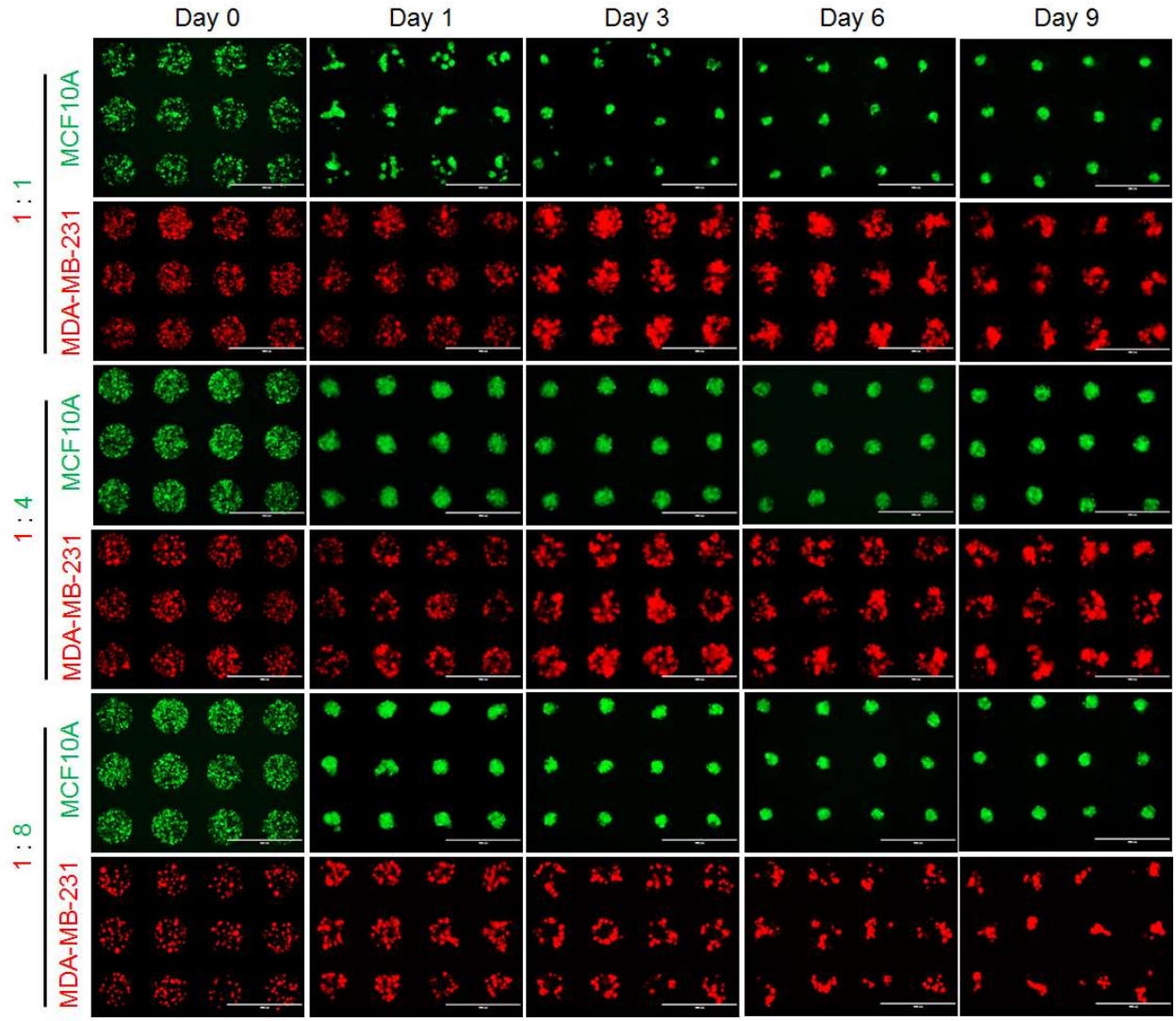

Figure S1. The respective fluorescent channels of growth-inhibited MDA-MB-231 (red color) and MCF10A (green color) cells in cell self-organization at different cell seeding ratios (1:1, 1:4, and 1:8; MDA-MB-231:MCF10A) during 9 days of culture in PDMS microwells of 200 μm diameter.

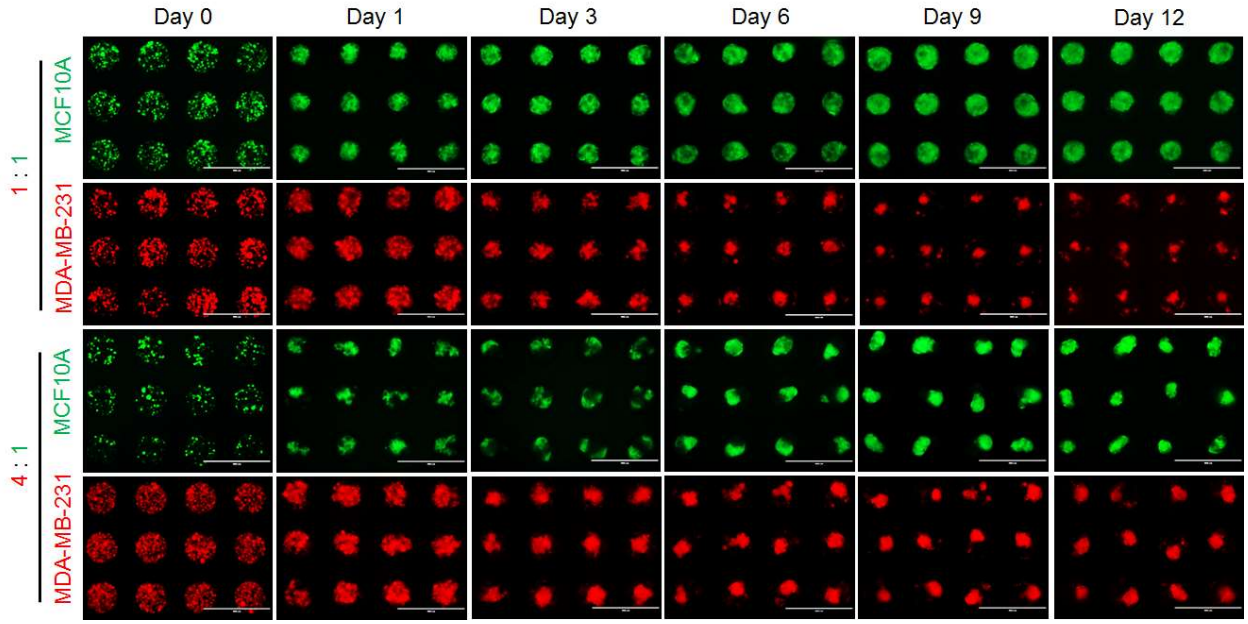

Figure S2. The respective fluorescent channels of growing MDA-MB-231 (red color) and MCF10A (green color) cells in cell self-organization at different cell seeding ratios (1:1 and 1:4; MDA-MB-231:MCF10A) during 12 days of culture in PDMS microwells of 200 μm diameter.

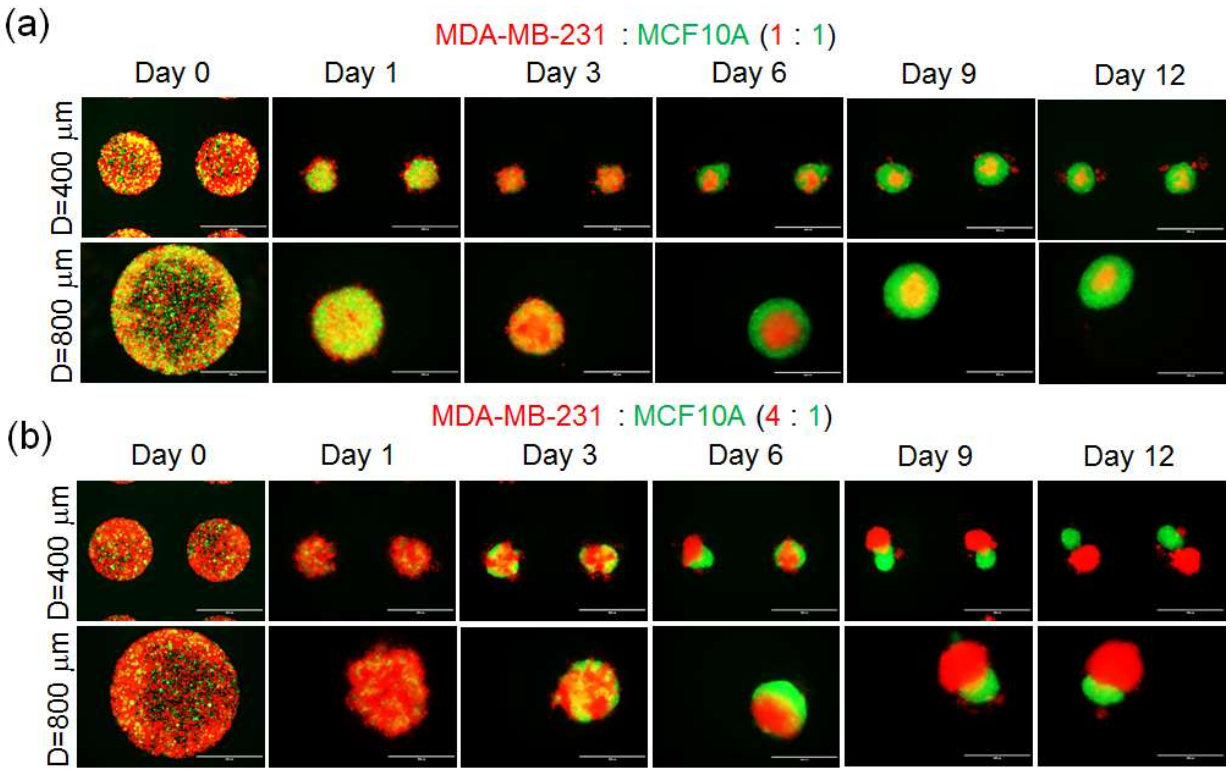

Figure S3. The evolvement of cell self-organization with proliferation during 12 days of culture in PDMS microwells of 400 and 800 μm diameters. The cell seeding ratios are 1:1 (a) and 4:1 (b). MDA-MB-231 cells are red color and MCF10A cells are green color.

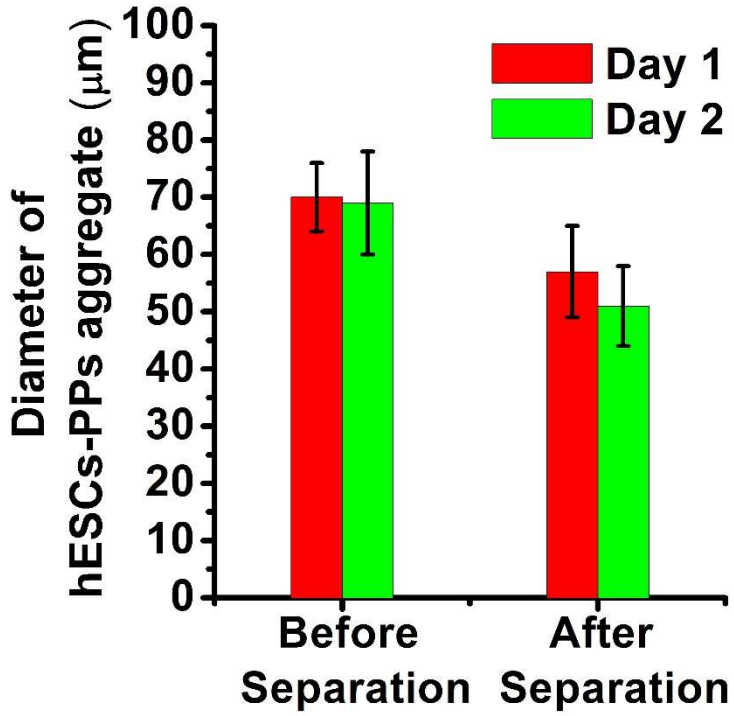

Figure S4. The diameter of hESCs-PPs aggregates before separation from MEF feeder cells and after separation during 2 days of culture in PDMS microwells of 200 μm diameter.

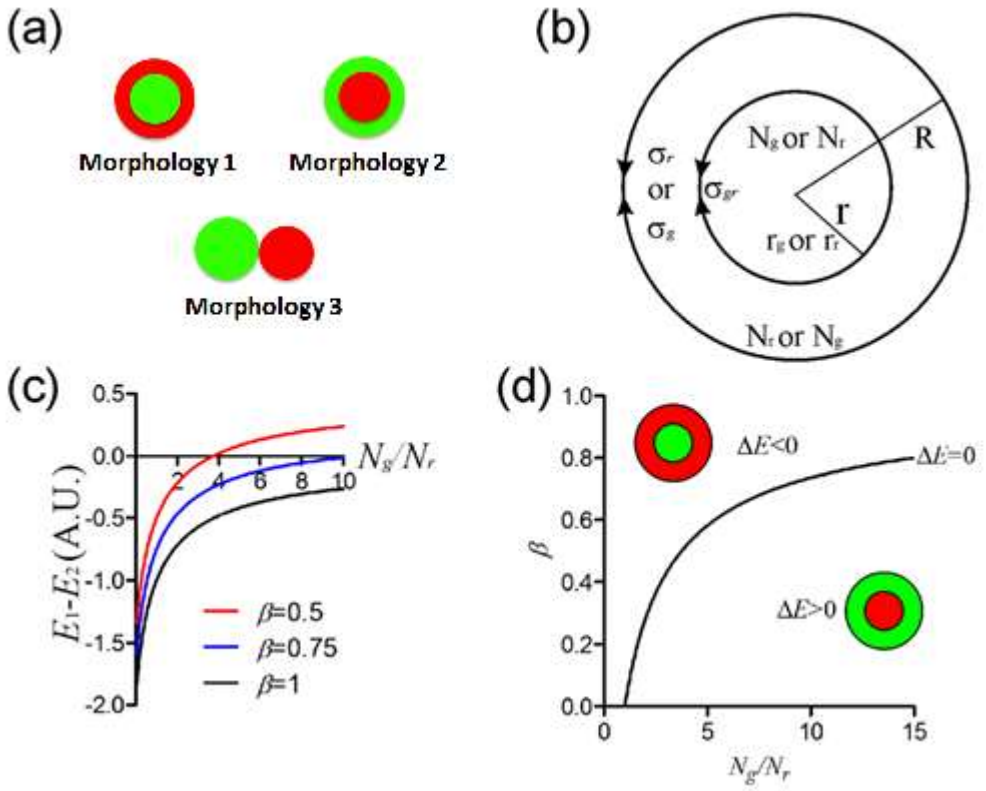

Figure S5. Free-energy calculation of different morphologies. (a) Schematics of three morphologies of the cell self-organization. (b) Definition of parameters for Morphology 1 and 2. (c) Free energy difference between Morphology 1 and 2. $E_1 - E_2$ is plotted in arbitrary unit. In all cases, $E_1 - E_2$ increases as N_g/N_r increases. Note $E_1 - E_2$ changes from negative to positive only when β is smaller than a critical value. (d) Phase diagram showing the borderline between two core-shell morphologies in $k - \beta$ space.

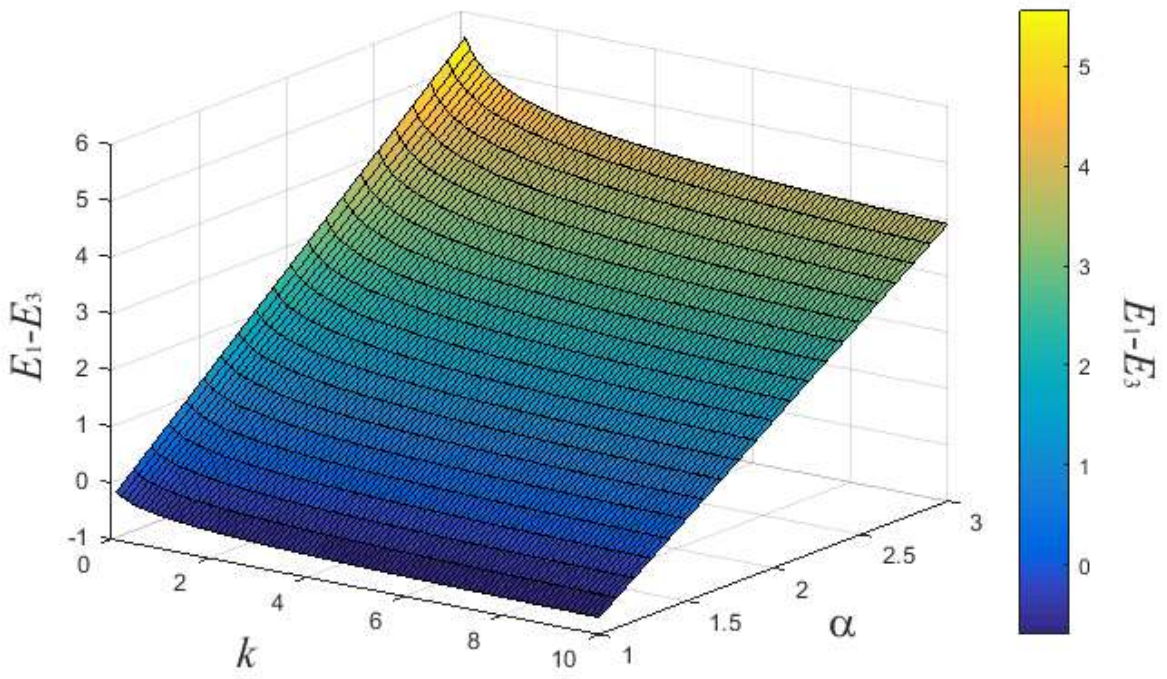

Figure S6. Free energy difference between core-shell and side-by-side morphologies for $\beta = 0.5$ case. Depending on the values of α and k, $E_1 - E_3$ can be either positive or negative. For the regions with $E_1 - E_3 < 0$, core-shell (morphology 1) is stable; otherwise the side-by-side (morphology 3) is more stable. Color code reflects $E_1 - E_3$ (shown on the right).