Supporting information

Effect of bioink properties on the printability and cell viability for 3D bioplotting of embryonic stem cells

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Shear stress determination

As stated in section "Materials and Methods", shear rate $\dot{\gamma}$, index K and n need to be determined to calculate shear stress by using Eq. (3).

Shear rate $\dot{\gamma}$ calculation

Assuming that the bioink is laminar fluid in the needle, the velocity demonstrates a parabolic distribution through the cross-section (Figure S1). Therefore, the share rate can be expressed as:

$$\dot{\gamma}(r) = \frac{dv(r)}{dr} = \frac{2v_{max}}{R^2} \cdot r \qquad \text{(S1)}$$

where, v_{max} is the velocity on the central axis, r is the radius from central axis, R is the inner radius of needle. The extrusion flux Q is a function of v_{max} as following,

$$\mathrm{Q} = \pi R^2 v_{avg} = \frac{\pi R^2 v_{max}}{2} \qquad \text{(S2)}$$

where, v_{avg} is the average velocity through the cross-section. From Eq. (S1) and (S2), the shear rate is thus given as:

$$\dot{\gamma}(r) = \frac{4Q}{\pi R^4} \cdot r \qquad \text{(S3)}$$

Q and R are known input parameters, which are 0.68 μL/s and 130 μm, respectively, as stated in section "Materials and Methods". Hence the maximum shear rate can be calculated as:

$$\dot{\gamma}_{max} = \frac{4Q}{\pi R^3} \approx 394\ (1/s)$$

Index K and n determination

According to power law of non-Newtonian viscosity, Eq (4) can be further logarithmically transformed to:

$$\lg(\eta) = \lg(K) + (n-1) \cdot \lg(\dot{\gamma}) \qquad \text{(S4)}$$

In a linear fitting for the relationship of $\lg(\eta)$ and $\lg(\dot{\gamma})$, $\lg(K)$ and $(n-1)$ are Y-intercept and slope, respectively. Therefore, index K and n can be obtained experimentally via viscosity curve in flow test as showed in Figure S2.

Basically, under certain shear rate, viscosity should be constant. However, Figure S3 (in time sweep flow test), together with Figure 3 (in time sweep oscillation test), indicated that the viscosity changed with time when the bioink was placed at different temperature from initial

temperature of 37°C. We assumed that the bioink got varying consistency index K during this process, while got constant flow behavior index n. Hence, in this study, n was obtained from viscosity curve, while K was obtained from time sweep tests under the same shear rate as printing by using Eq. (S5):

$$K(t) = \eta(t) \cdot \dot{\gamma}^{1-n} \qquad \text{(S5)}$$

which was transformed from Eq. (4).

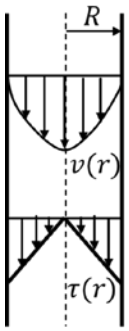

Figure S1. Distribution of laminar fluid velocity ($v(r)$) and shear stress ($\tau(r)$) through the cross-section of needle.

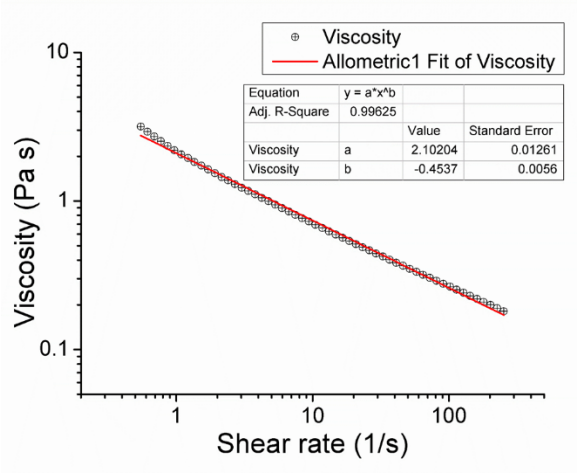

Figure S2. A representative linear fitting of logarithmical relationship between viscosity and shear rate. Biolink of 5%Gel+1%Alg was used under the temperature of 27.5°C.

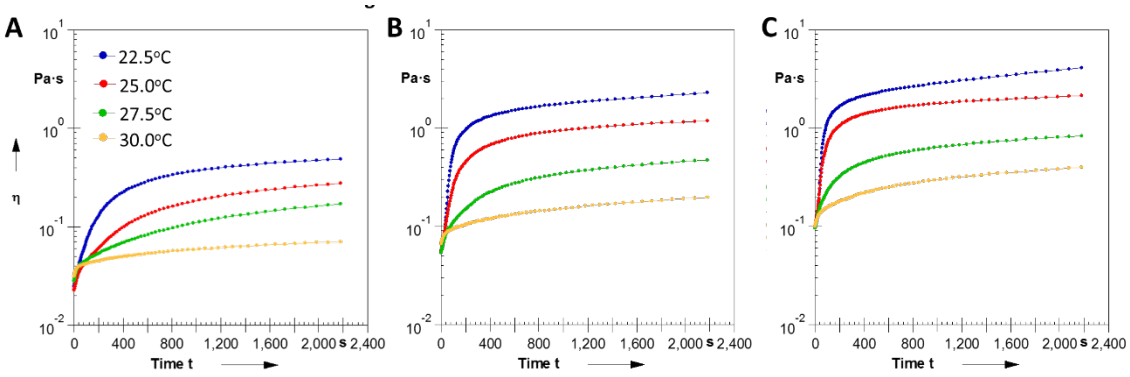

Figure S3. Time sweeps flow test under different temperature for (A) 5%Gel+1%Alg, (B) 7.5%Gel+1%Alg, and (C) 10%Gel+1%Alg. Shear rate was fixed as 394 (1/s).

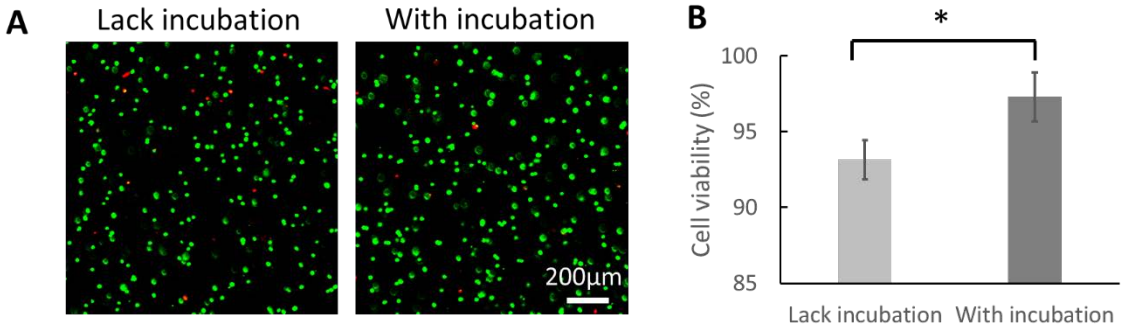

Figure S4. The difference on cell viability of tow protocols for blending cells with hydrogel: (A) Images of live/dead staining and (B) quantified viability. In "Lack incubation" group, cell suspension (RT) were blended with alginate and gelatin successively at RT, which took around 2min. In "With incubation group", dissolved gelatin and alginate solutions were blended firstly and incubated at 37 °C. After incubation at 37 °C, cell suspension was added to the blended hydrogel quickly (within 1min).

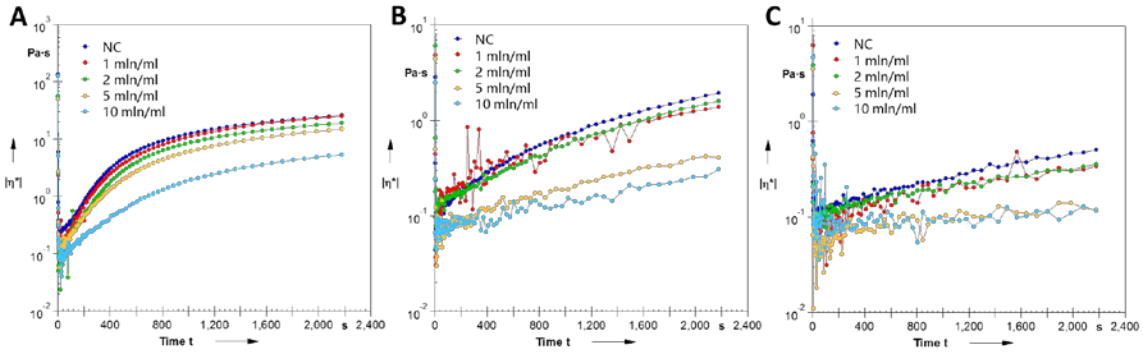

Figure S5. Time sweeps oscillatory test under different cell concentration at (A) 25 °C, (B) 27.5 °C and (C) 30 °C. Bioink of 7.5%Gel+1%Alg was used under strain of 0.1% and frequency of 1.5 Hz. The control group (NC) was bioink without cells.

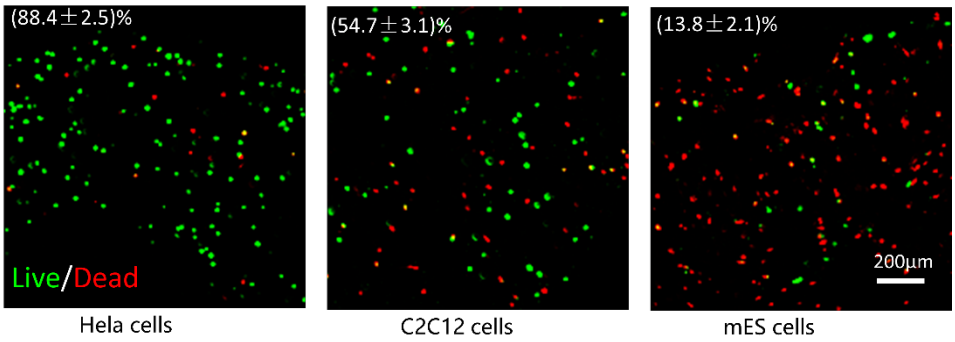

Figure S6. Cell viability varies among different cell types under the same printing parameters (7.5%Gel+1%Alg with 1×10^6 cells/ml at PT-22.5 ºC and time is ~10 min).

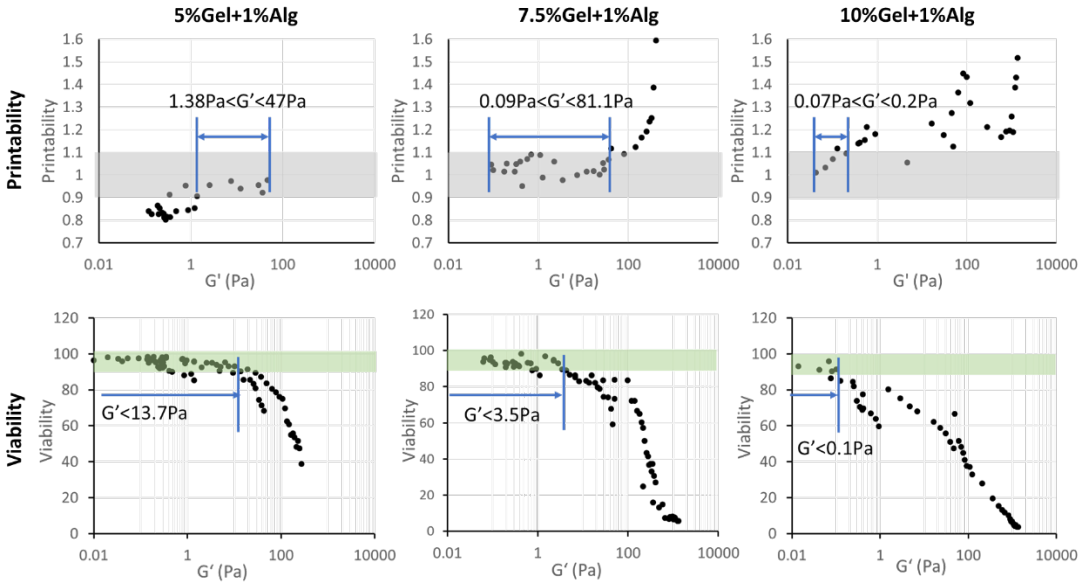

Figure S7. Printability and viability act as a function of G' under certain bioink concentration

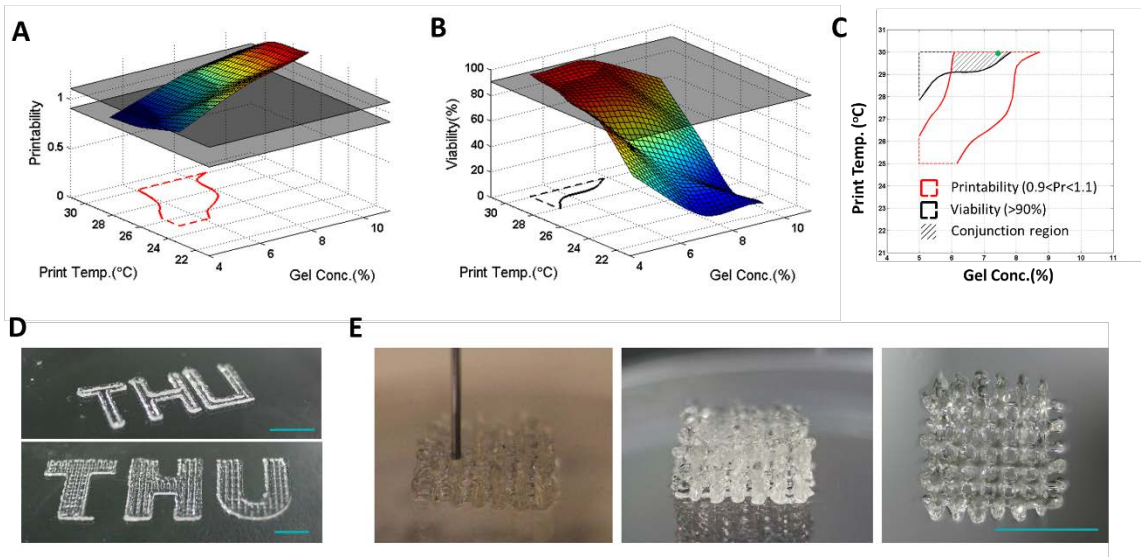

Figure S8. The balance of printability and viability for basic parameters of gelatin concentration and printing temperature at 30 min. (A) 3D surface showed printability as a function of gelatin concentration and printing temperature and a proper range of printability from 0.9 to 1.1 was set to determine the printability region. (B) 3D surface showed viability as a function of gelatin concentration and printing temperature and viability of more than 90% was set to determine the viability region. (C) The cross section of printability and viability regions determined the balance region (shaded area). (D) Representative images of printed one-layer (up) and two-layer (down) “THU” logo. (E) Different views of a 20-layer grid structure. Both (D) and (F) were applying the optimized parameters configuration within the balance region (green spot) in (C). Scale bars are 5 mm.