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Monodisperse collagen/gelatin beads as potential platforms for 3D cell culturing

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Supplementary Information

1. Mechanical characterization

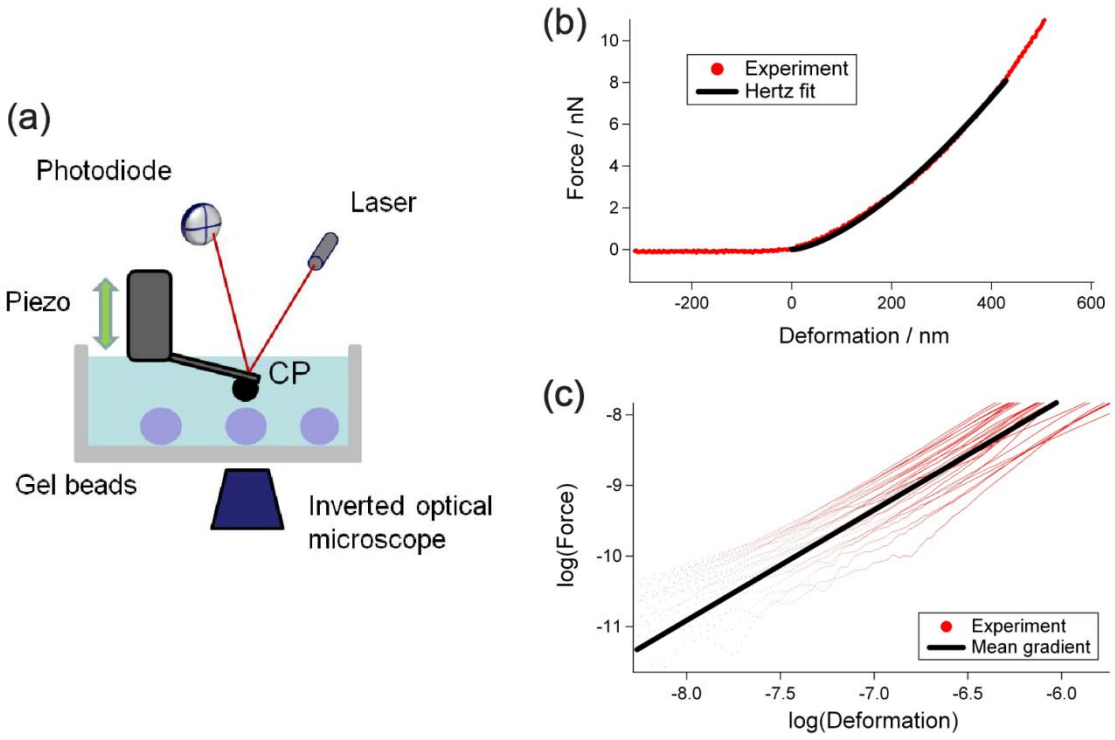


Figure 1: (a) Illustration of micromechanical characterization of gelatin and col/gela microbeads using AFM combined with an inverted optical microscope. The colloidal probe (CP) with a glass bead rather than a sharp tip is forced on a gel bead, which subsided to the cell bottom, immersed in water. Bead alignment and gel bead deformation is monitored from the microscope. (b) A typical force-deformation curve with Hertz fit. (c) Scatter plot of randomly selected force curves proving the Hertzian scaling law.

2. SEM characterization

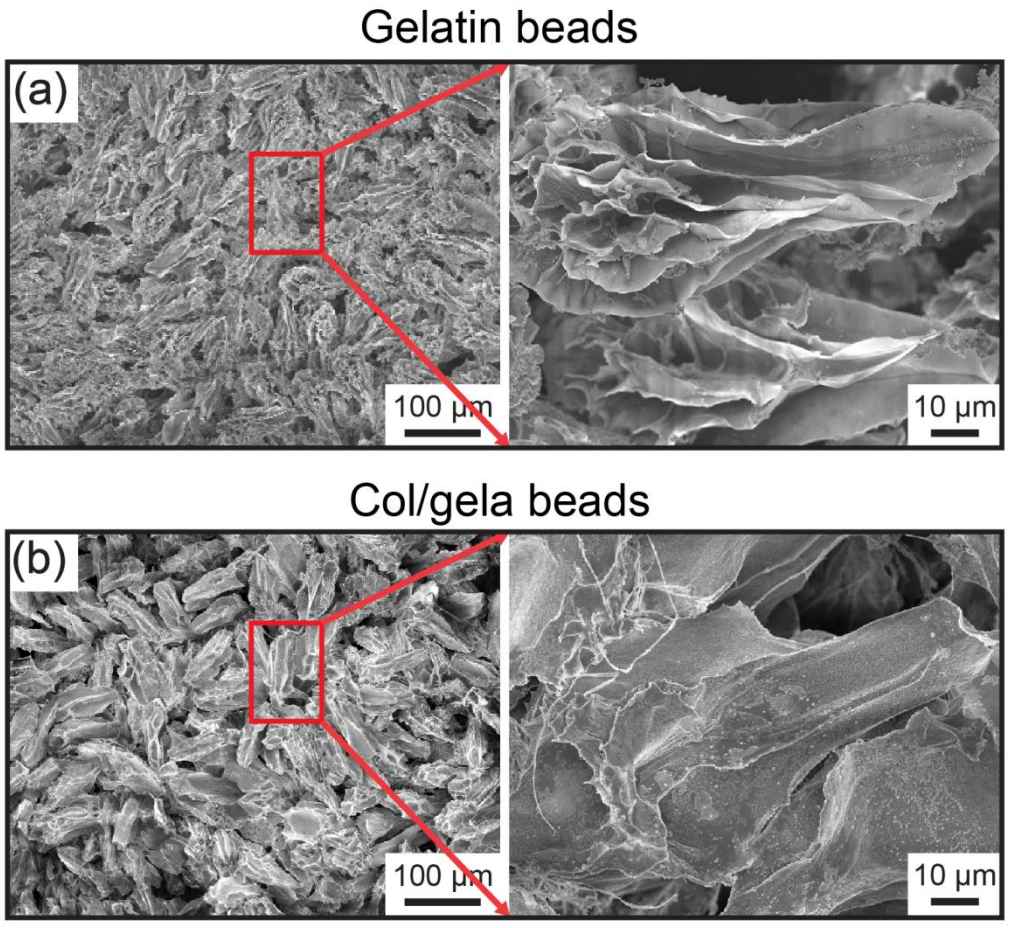
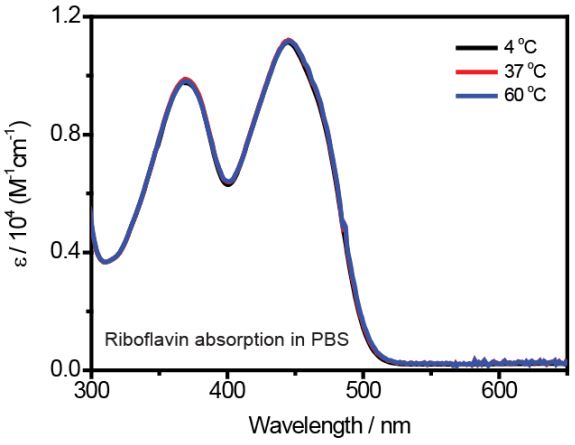


Figure 2: SEM images of gelatin beads (a) and col/gela beads (b). Beads are (a) 4.0 wt% gelatin, 4.5 min crosslinking, freeze-dried and (b) 4.0 wt% gelatin, 0.18 wt% collagen, 4.5 min crosslinking, freeze-dried.

3. Crosslinking and degradation parameters

(a)

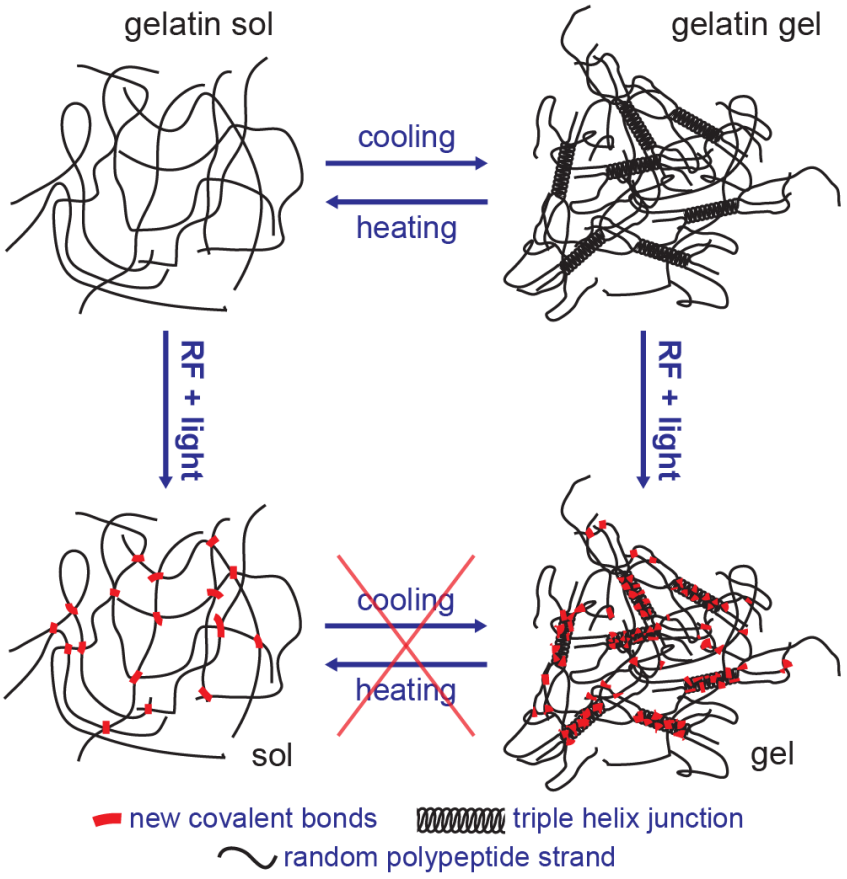


(b)

Crosslinking temperature	37 °C incubation time		
	1 h	24 h	48 h
4 °C	Spherical beads	Spherical beads	Spherical beads
37 °C	Sticky, deformed beads	Sticky, deformed beads	Viscous solution
60 °C	Viscous solution	Non-viscous solution	Non-viscous solution

Figure 3: (a) Adsorption spectrum of riboflavin in PBS buffer at three different temperatures: 4 °C, 37 °C, and 60 °C. The adsorption curves have no obvious difference, indicating that in these conditions the crosslinking activity remains unchanged. (b) Morphological evolution of 4.0 wt% gelatin beads crosslinked at 4 °C, 37 °C, and 60 °C for 4.5 min, respectively and incubated at 37 °C oven 48 h in PBS buffer.

4. Photo-crosslinking mechanism



Scheme 1: Sol-gel phase transition of gelatin before and after riboflavin crosslinking. By cooling or hearting, gelatin transit between sol and gel phases before photocrosslinking; but after crosslinking, the sol-gel transition is prohibited.

5. FITC-dextran hydrodynamic radius

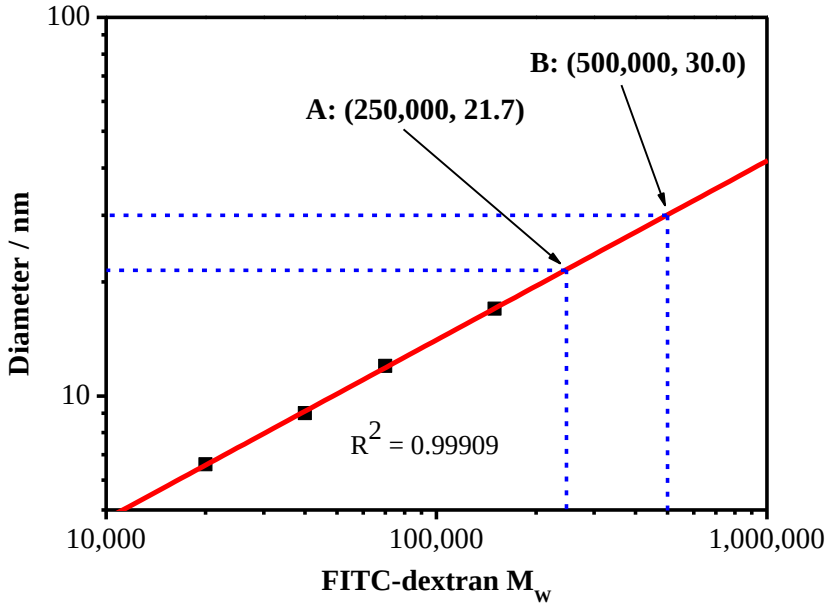


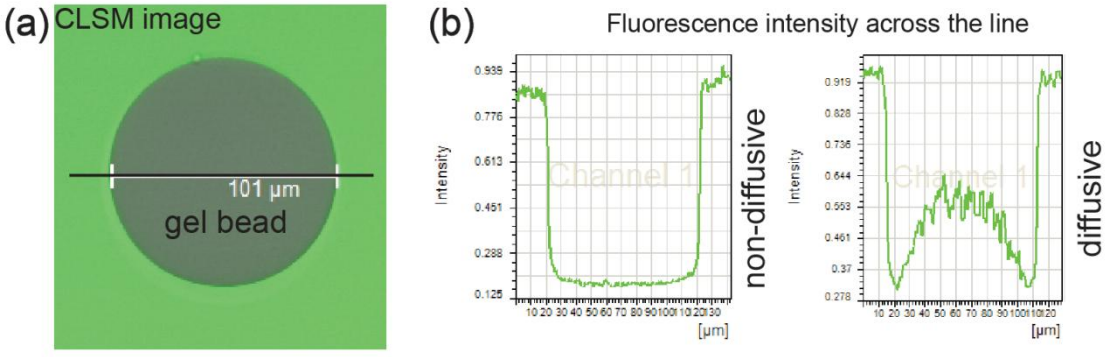
Figure 4: Log-log plot of hydrodynamic diameter against different molecular weights, $M_{W,}$ of FITC-dextran in PBS buffer. The plot is fitted linearly (adjusted R^2 = 0.99909), in agreement with the predicted power relationship of the hydrodynamic radius, R_h, with the number of monomers, N, in a polymer chain, $R_h \propto N^{v}$.[1] The exponent, v, is determined by the polymer and the solvent.

	Molecular weight, M_W	Hydrodynamic diameter D_h (nm)
Data from *Supplier*	20,000	6.6
	40,000	9.0
	70,000	12.0
	150,000	17.0
Data from fitted line	250,000	**21.7**
	500,000	**30.0**

Table 1: Hydrodynamic diameters of FITC-dextran of different Mw

The hydrodynamic diameters of FITC-dextran is obtained from *Product Information* from *Sigma-Aldrich*, of molecular weights *of 20,000*, *40,000*, *70,000*, and *150,000*.[2] In literatures the log-log plot of R_h against M_W has been demonstrated linear from M_W = *10,000* to *2000,000*, and the hydrodynamic diameters read from the fitted line is listed in table S1 and matches extraordinarily well with the reported data .[3,4]

6. Mesh size measurement via FITC-dextran diffusion



(c)

Composition (wt)	Mesh size (nm)	Composition (wt)	Mesh size (nm)
1.5% gela	17.0 – 21.7	1.5% gela + 0.18% col	> 30.0
2.0% gela	17.0 – 21.7	3.0% gela + 0.18% col	21.7 – 30.0
3.0% gela	17.0 – 21.7	5.0% gela + 0.18% col	17.0 – 21.7
10.0% gela	17.0 – 21.7	7.5% gela + 0.18% col	17.0 – 21.7

Figure 5: (a) CLSM image of a gelatin bead immersed in FITC-dextran (150S) PBS solution; (b) normalized quantified fluorescence across the line in (a) of a 'non-diffusive' bead and a 'diffusive' bead; (c) list of mesh size of gelatin and col/gela beads at different gelatin concentrations.

Mesh size is a critical parameter in biodegradable hydrogels as it determines the diffusion of nutrients, grow factors, metabolites and facilitates cell migration.[5,6] Preliminary characterisation of the mesh size in gelatin beads is carried out by measuring the

fluorescence diffusion of FITC-dextran at different molecular weights ranging from 40,000 to 500,000 (abbreviated as 40S and 500S, respectively) corresponding to hydrodynamic diameters between 9.0 nm and 30.0 nm (Figure 5 and Table 1). The mesh sizes were estimated by determining the cut-off size at which dextran no longer diffused into the gel beads. As shown in Figure 6c, all gelatin beads concentrated from 1.5 wt% to 10.0 wt% of gelatin gave mesh size in the range of 17.0 nm to 21.7 nm. The addition of collagen increased the mesh size to 21.7 - 30.0 and > 30.0 nm for beads containing 3.0 and 1.5 wt% gelatin, respectively, which is attributed to larger mesh size, in microns range, of the collagen matrix.[7]

7. Swelling ratio

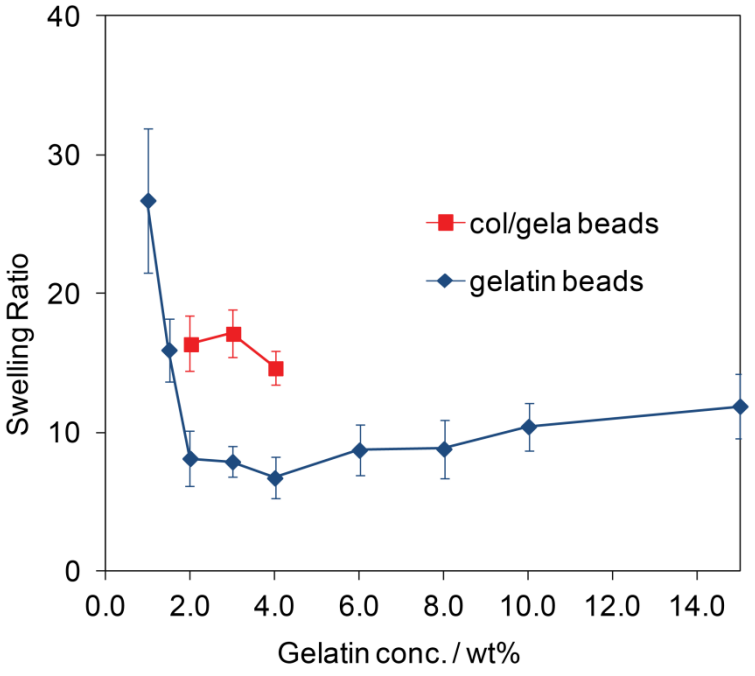


Figure 6: Swelling ratios of gelatin beads and col/gela beads at different gelatin concentrations. Beads were crosslinked for 4.5 min and blended with 0.18 wt% collagen.

8. Flory-Rehner Theory

Based on the swelling theory originally developed by Flory and Rehner[8] we calculated the mesh size of gelatin hydrogel beads using the following equations. Generally, the molecular weight between two adjacent crosslinks, $\bar{M}_c$, can be related to the molecular weight of the polymer, $\bar{M}_n$, and the polymer volume fraction in swollen state, $v_{2,s}$ (the inverse of the swelling ratio), as

$$\frac{1}{\bar{M}_c} = \frac{2}{\bar{M}_n} + \frac{\bar{v}}{V_1} \cdot \frac{ln(1-v_{2,s}) + v_{2,s} + \chi \cdot v_{2,s}^2}{v_{2,s}^{1/3} - v_{2,s}/2}$$

with $\bar{v}$ being the specific volume of the polymer, V_1 the molar volume of water and χ the Flory-Huggins interaction parameter. The mesh size, ξ, can be obtained from

$$\xi = v_{2,s}^{-1/3} \cdot \left(\frac{2\, C_n\, \bar{M}_c}{M_r}\right)^{1/2} \cdot l$$

Here, C_n is the Flory characteristic ratio, M_r the molecular weight of the repeating units and l the length of the bond along the polymer backbone.[9] For large chains C_n equals a limiting value C_∞, which can be calculated from the persistence length, l_p:

$$l_P = (C_\infty + 1) \cdot \frac{l_s}{2}$$

Here, l_s is the linear segment length.[10]

Since the Flory-Rehner theory as presented above is strictly valid only for simple systems like vinyl polymers, some modifications have to be made and constants are to be chosen with care. First, the factor 2 in equation (2) has to be replaced by factor 3 because the repetitive unit already consists of 2 bonds (instead of 1 like for vinyl polymers). Second, l is

the arithmetic mean of one C-C bond and two C-N bonds. Third, M_r is the mean molecular weight as calculated on the basis of the typical amino acid composition of the used gelatin. Last, the segment length is taken as the sum of one C-C bond and two C-N bonds. All values are listed in Table 2.

Constant	**Abbreviation**	**Value**	**Reference**
Specific volume	$\bar{v}$	0.741 cm³/g	11
Flory-Huggins parameter	χ	0.497	11
Molecular weight of repeating unit	M_r	94.7 g/mol	Calculated after ref. 11
Persistence length	l_p	20 Å	11
C(carbonyl)-C bond length	-	1.53 Å	12
C(carbonyl)-N bond length	-	1.32 Å	12
C-N bond length	-	1.47 Å	12
Flory characteristic ratio	C_∞	8.26	Calculated

Table 2: Parameters and given values in above equations

The value for C_∞ we calculate for gelatin compares well with characteristic ratio values for other polypeptides reported in literature.[13]

The overview of the swelling ratios and therefore the calculated mesh for a fixed crosslinking time (4.5 min) is given in the main text Tabel 1.

The Flory-Rehner theory assumes a rather ideal picture of crosslinked polymers. Yet, we find good agreement with experimentally determined mesh size over a large range of

concentrations. This could be an effect of the presence of triple-helices. Their amount was reported to be almost independent of concentration between 2 and 8 % of gelatin.[14] The helices introduce order into the network making it more comparable to the theoretical model. For very low concentrations close to or even below the gelling limit of gelatin[15,16] only few or no helices are formed. Therefore, swelling is enhanced but the experimentally determined mesh size is more influenced by entanglements, loops or dangling chains which accounts for the discrepancy with the theoretical values.

9. 3T3 fibroblast viability compartmentalized in col/gela beads

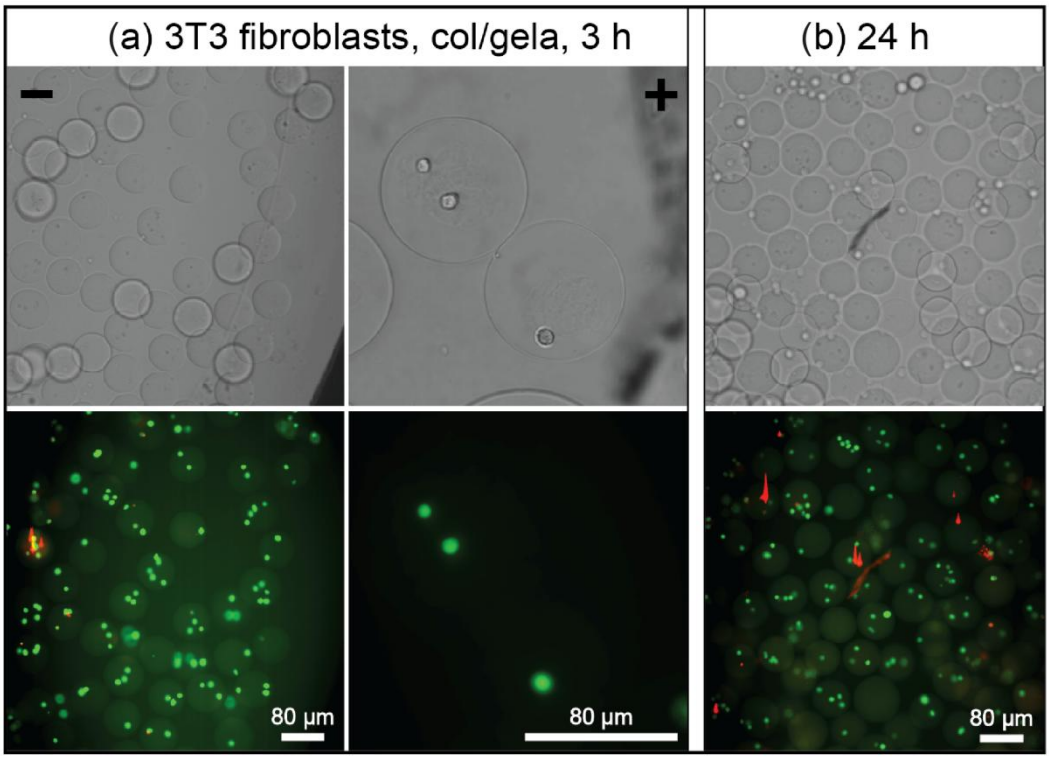


Figure 7: Bright field images (above) and the corresponding fluorescence images (below) of 3T3 fibroblasts in col/gela beads (4.0 wt% gelatin with 0.18 wt% collagen, 8.0 min crosslinking, 2.6×10^6 cells / ml) over 24 hrs. Cells were stained with *Live/Dead Viability/Cytotoxicity kit*, indicating live (green) and dead (red) cells after different culture time.

Reference

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