Supporting Information

The increase in the pore size of Gelatin Methacryloyl (GelMA) macroporous hydrogel promotes cell proliferation by enhancing substance permeability

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This PDF file includes:

Supplementary Methods

I A Specific Method for Solving the Effective Diffusion Coefficient Using Classical Analytical Solutions Based on Fick's Second Law

1. Physical Model Assumptions

In my Transwell experiment, the diffusion system is considered to be one-dimensional diffusion:

2. Diffusion Equation

For one-dimensional unsteady-state diffusion [1,2]:

$$\frac{dC}{dt} = D_{eff}\frac{d^2C}{dx^2}$$

Where $C(x,t)$ is the concentration ($mol \cdot L^{-1}$) at position x inside the hydrogel at time t, D_{eff} is the effective diffusion coefficient (cm^2/s), t is time (s), and x is the spatial coordinate ($0 \leq x \leq L$, unit: cm).

3. Initial and Boundary Conditions

Initial Condition (t=0)	Boundary Condition 1 (x=0)	Boundary Condition 2 ($x = L$)
$C(x,0) = 0$	$C(0,t) = C_0$	$C(L,t) = 0$
(No fluorescent substance inside the hydrogel)	(Interface concentration at the upper chamber is C_0)	(Interface concentration at the lower chamber is 0)

4. Model Solving

The classical analytical solution for Fick's diffusion [3,4] is used to solve the model and obtain the relationship between cumulative permeation M_t and time t:

$$C(x,t) = C_0\left(1 - \frac{x}{L}\right) - \frac{2C_0}{\pi}\sum_{n=1}^{\infty}\frac{1}{n}\sin\left(\frac{n\pi x}{L}\right)exp\left(-\frac{D_{eff}n^2\pi^2 t}{L^2}\right)$$

The mass M_t accumulated in the lower chamber per unit area is:

$$M_t = A\int_0^t (-D_{eff}\frac{dC}{dx}|_{x=L})dt$$

After integration, we obtain:

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\sum_{n=0}^{\infty}\frac{1}{(2n+1)^2}exp\left[-\frac{D_{eff}(2n+1)^2\pi^2 t}{L^2}\right]$$

Where M_∞ is the total permeation amount in the lower chamber after infinite time. In my Transwell experiment, since the lower chamber is continuously replaced, M_∞ should theoretically be the total mass initially in the upper chamber.

5. Fitting Based on Existing Experimental Data Using Classical Analytical Solutions

5.1 Data Preparation

Extract data points (Time t, Cumulative Permeability %) from Figures 4C, E, and G.

Convert Cumulative Permeability % into the actual mass permeation M_t

$$M_t = \frac{Cumulative\ Permeability}{100} \times M_0$$

Where $M_0 = C_0 \times V_{upper}$ (Initial total mass in the upper chamber).

5.2 Defining the Fitting Function

Using Origin Pro 2021 software to create a new function

5.2.1 Open the Fitting Function Builder

Menu: Analysis → Fitting → Nonlinear Curve Fit → Open Dialog Box

5.2.2 Create a Custom Function

① Select the **Function** tab in the dialog box.

② Click **New** → Select **New Function**

③ Set function name and type:

Function Name	Function Type	Independent	Dependent Var	Parameter

		Var		Names
FickSecondLaw	User Defined	t(Time, unit: seconds)	M_t(Cumulative penetration, unit: μg)	a) Deff: Effective diffusion coefficient (cm^2/s) b) Minf: Total permeation over infinite time (μg) c) L: Hydrogel thickness (cm)

5.2.3 Input Function Expression

Enter the following code in the Function edit box (C language format):

```
// Variable Definition
double sum = 0;
double term;
int n;
int n_terms = 50;    // Number of terms in a series

// Calculate the sum of a series
for(n = 0; n < n_terms; n++)
{
    term = 1.0 / ((2*n+1)*(2*n+1)) * exp(-Deff * (2*n+1)*(2*n+1) * pi * pi * t / (L*L));
    sum += term;
}
```

```
// Calculate M_t
Mt = Minf * (1 - 8/(pi*pi) * sum);
```

5.2.4 Set Initial Parameter Values

Set in the **Parameters** tab:

Deff	Minf	L
5×10^{-10} (Initial guess value)	10(M_0 = 10 μg)	0.1(Hydrogel thickness: 1 mm)

5.2.5 Save Function

Click **Save** to store the function in the **User Defined** category.

5.3 Executing Nonlinear Fitting

5.3.1 Select Data and Function

① Add a new column Time_s = Time_h * 3600 to the data table.

② Select the data columns to be fitted in the worksheet (e.g., Time_s and M_t_GelMA).

③ Reopen the Nonlinear Fit dialog box(Analysis → Fitting → Nonlinear Curve Fit → Open Dialog Box)

④ In Settings → Function Selection:

Category	Function
User Defined	FickSecondLaw

5.3.2 Set initial values, fixed conditions, and constraints

On the **Parameters** tab:

Parameters	Initial value	Is it fixed?	Constraint
Deff	5×10^{-10}	No (non-fixed)	0<Deff< e-8
Minf	10	Yes (fixed)	-
L	0.1	Yes (fixed)	-

5.3.3 Perform Fitting

Click the **Fit** button, and Origin will:

① Iteratively compute optimal parameters

② Generate a fitted curve

③ Output the fitting report

5.4 Add confidence intervals to plots with generated fitted curves

5.4.1 Reopen the Fit Dialog Box

In the graph where the fitted curve has already been generated, click the green lock icon on the graph, then click “Change Parameters” to reopen the “Nonlinear Curve Fitting (NLFit)” dialog box.

5.4.2 Locate the “**Settings**” tab

In the row of tabs in the middle of the dialog box, click “**Settings**.”

5.4.3 Accessing “**Fitted Curves**”

In the list on the left side of the “Settings” tab, click “**Fitted Curves**”.

5.4.4 Check the Confidence Band

In the menu that expands on the right, locate the “Fitted Curves Plot” section. Check the box:

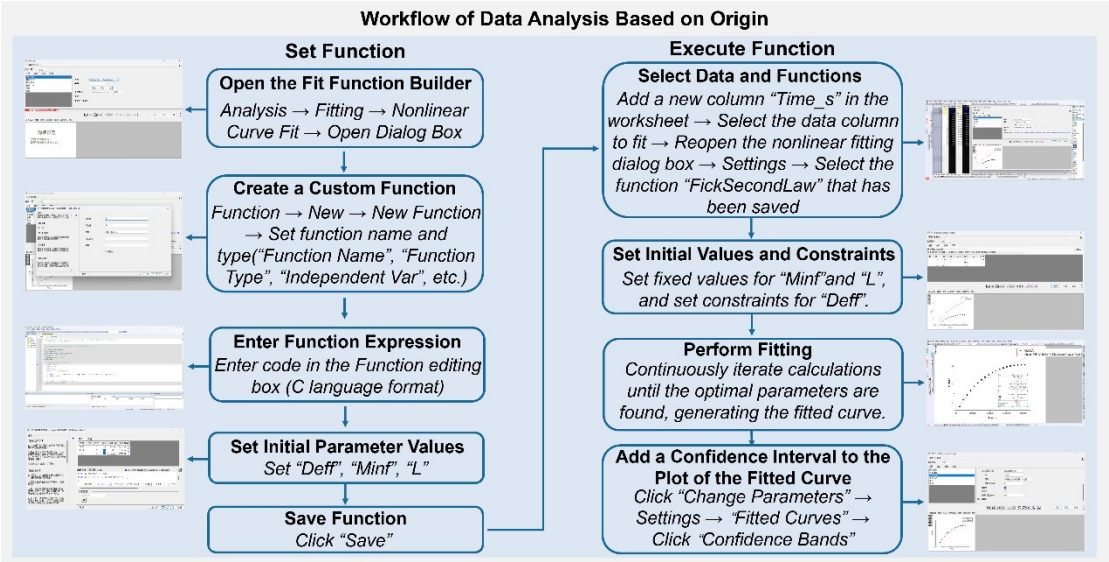
① Confidence Bands: Checked, with a default setting of 95%.

② Prediction Bands: Checked, with a default setting of 95%.

5.4.5 Click “Fit”

After completing the settings, click the “Fit” button at the bottom.

5.5 Adjust the horizontal time axis and vertical cumulative permeability axis using mathematical formulas to match Figure 4 in the manuscript.



Supplementary Method Figure 1 Use Origin software to build and execute the classical analytical solution function of Fick’s second law.

II A Specific Method for Normalizing Permeability to the Average Pore Size

1. Calculate the free diffusion coefficient D_0 using the Stokes-Einstein equation.

Calculate D_0 according to the Stokes-Einstein equation [5]:

$$D_0 = \frac{k_B T}{6\pi\eta R_h}$$

k_B(Boltzmann constant)	T (Temperature)	η (PBS Viscosity)	R_h(Hydrodynamic radius)
$1.38 \times 10^{-23} J/K$	298.15K	$0.89 \times 10^{-3} Pa \cdot s$	**BSA:** 3.5 nm [6] **Dextran:** 1.4 nm [7] **Mannose:** 0.6 nm [8]

$D_0(cm^2/s)$	BSA	Dextran	Mannose
	6×10^{-7}	1.7×10^{-6}	5.4×10^{-6}

2. Obtaining aperture dimensions using ImageJ software

2.1 Importing CLSM Images into ImageJ Software

2.2 Set Scale

Select Analyze →Set Scale from the **menu bar**. Use the line tool to draw a ruler segment of known length on the image. In the pop-up window, enter the actual length and unit (e.g., μm). Check **Global** to apply the scale to all images.

2.3 Drawing Survey Lines

Select the Line tool and draw a line at each end of the hole to indicate its diameter.

2.4 Conducting Measurements

Select Analyze → Measure from the **menu bar**. In the results window, the **Length** column displays the measured diameter values.

2.5 Result Processing

Convert diameter to radius ($Mean_{r_p}$).

3. Use Origin software to plot scatter plots for all data and fit power-law trendlines.

3.1 Import the aperture size data obtained from ImageJ software into Origin.

Select the $Mean_{r_p}$ column (average pore size column) and the y_{exp} column (experimentally obtained $\frac{D_{eff}}{D_0}$), where D_{eff} is the effective diffusion coefficient calculated using the classical analytical solution based on Fick's second law.

3.2 Plotting Scatter Plots

Click the menu: Plot →Symbol →Scatter

3.3 Open the Nonlinear Fit dialog box

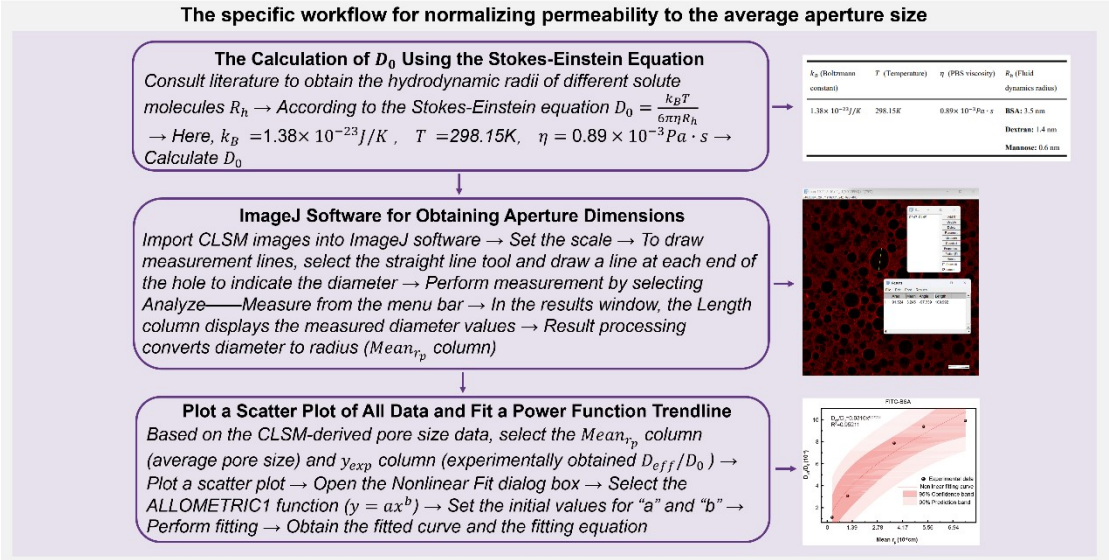
3.4 Select the ALLOMETRIC1 function ($y = ax^b$)

3.5 Set the initial values for "a" and "b"

Set a=1 and b=0.5 in the **Parameters** tab.

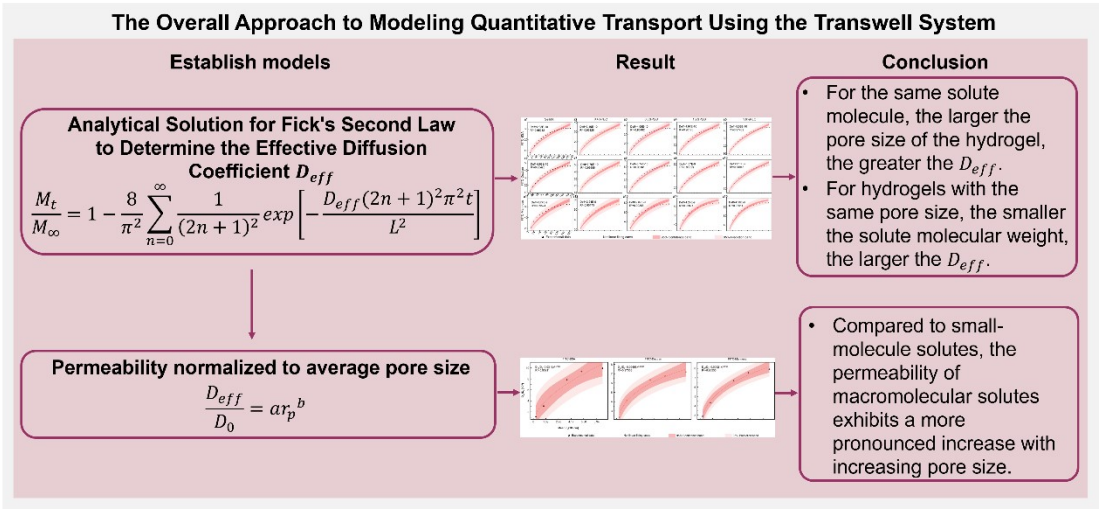
3.6 Perform fitting

Click the "**Fit**" button to perform the fit, obtaining the fitted curve and the fitting equation.



Supplementary Method Figure 2 The specific workflow for normalizing permeability to the average aperture size.

Supplementary Method Figure 3 demonstrated our overall approach to modeling quantitative transport using the Transwell system.



Supplementary Method Figure 3 The overall approach to modeling quantitative transport using the Transwell system.

Supplementary Figures 1-27

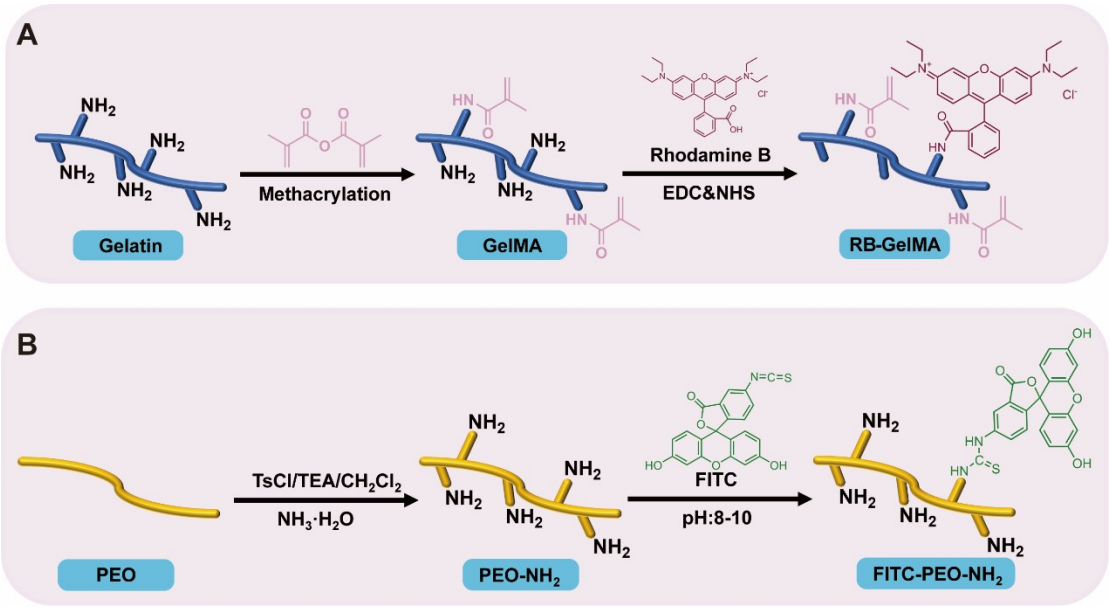


Fig. S1. A), Schematic diagram of the synthesis of RB-GelMA. B), Schematic diagram of the synthesis of FITC-PEO-NH_2.

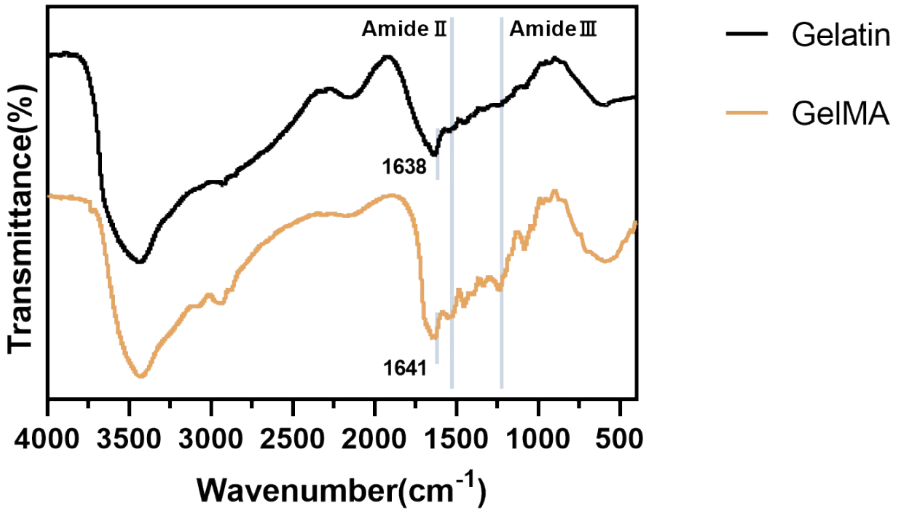


Fig. S2. FTIR spectra of GelMA

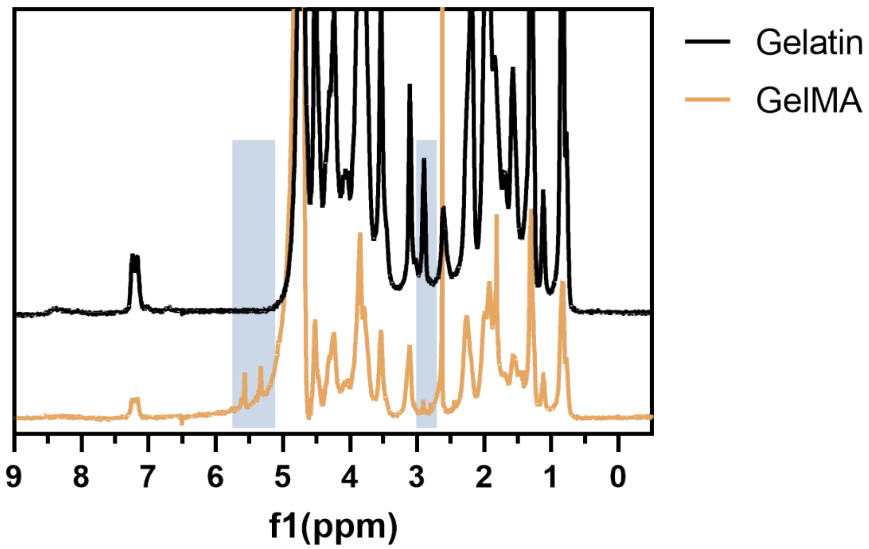


Fig. S3. Proton nuclear magnetic resonance spectroscopy of GelMA

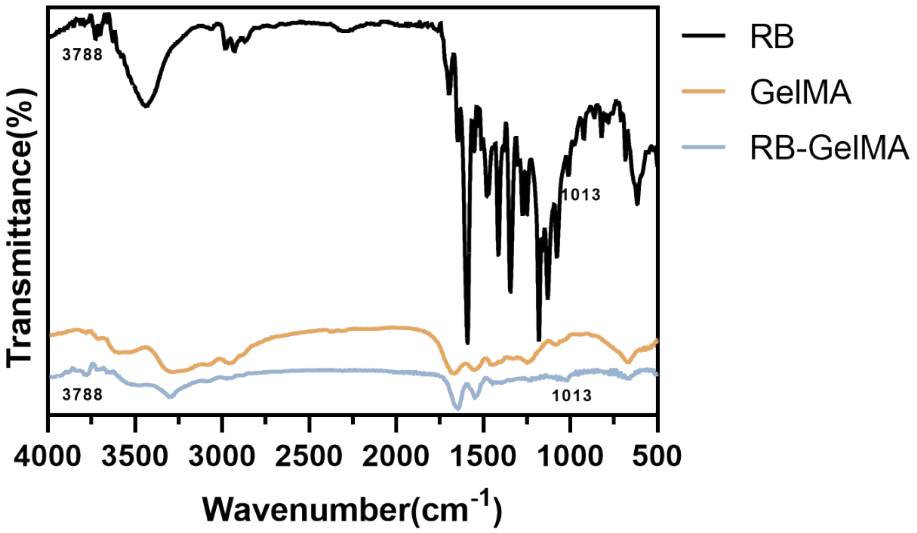


Fig. S4. FTIR spectra of RB-GelMA

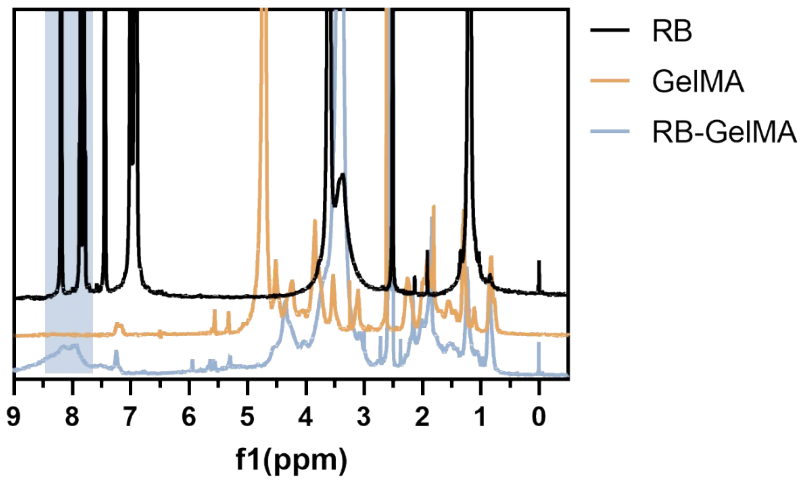


Fig. S5. Proton nuclear magnetic resonance spectroscopy of RB-GelMA

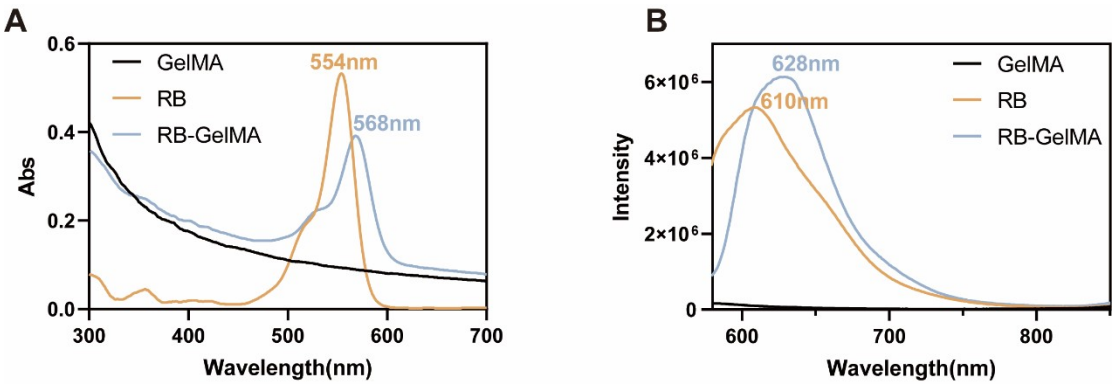


Fig. S6. A), UV-vis spectrum of RB-GelMA. B), Fluorescence spectrum of RB-GelMA.

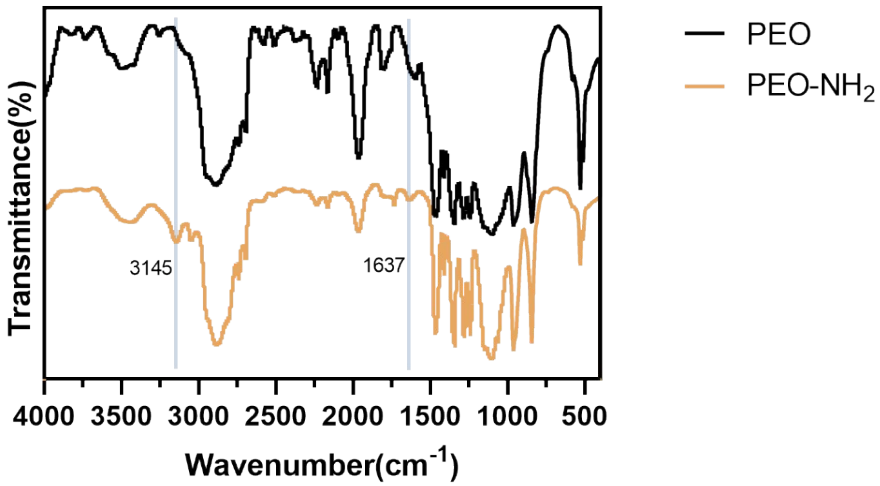


Fig. S7. FTIR spectra of PEO-NH_2

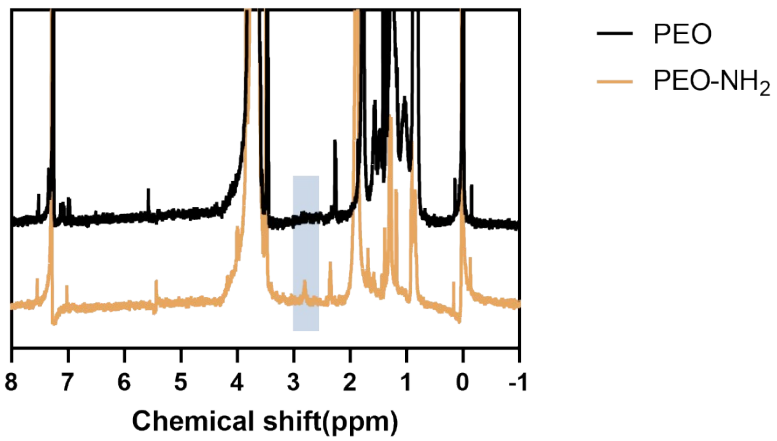


Fig. S8. Proton nuclear magnetic resonance spectroscopy of PEO-NH_2

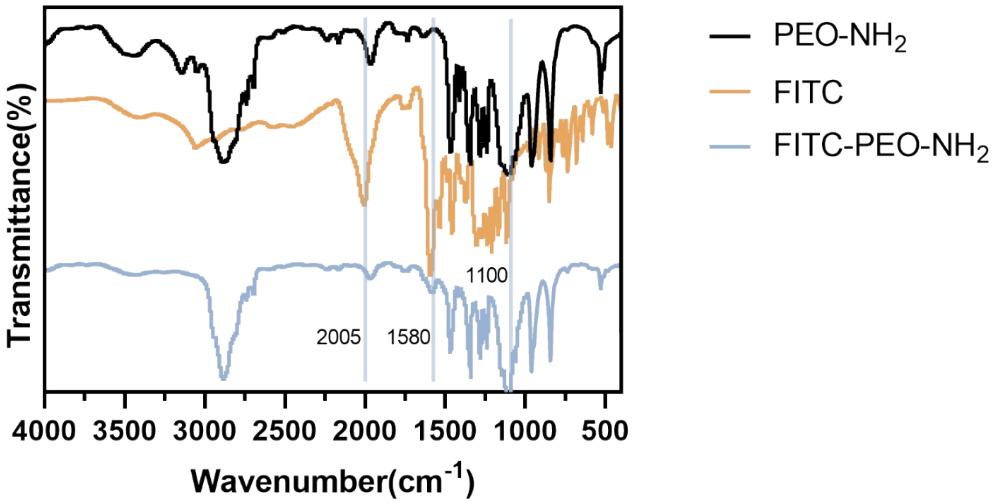


Fig. S9. FTIR spectra of FITC-PEO-NH_2

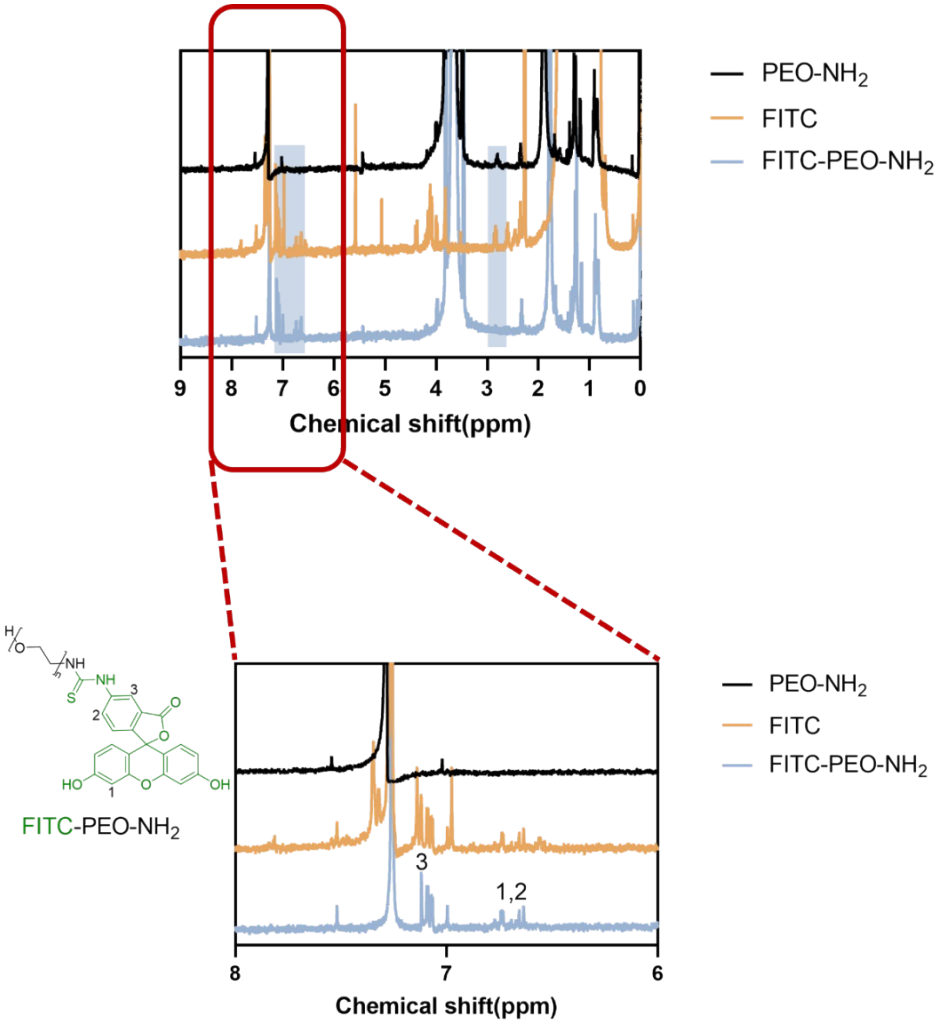


Fig. S10. Proton nuclear magnetic resonance spectroscopy of FITC-PEO-NH_2

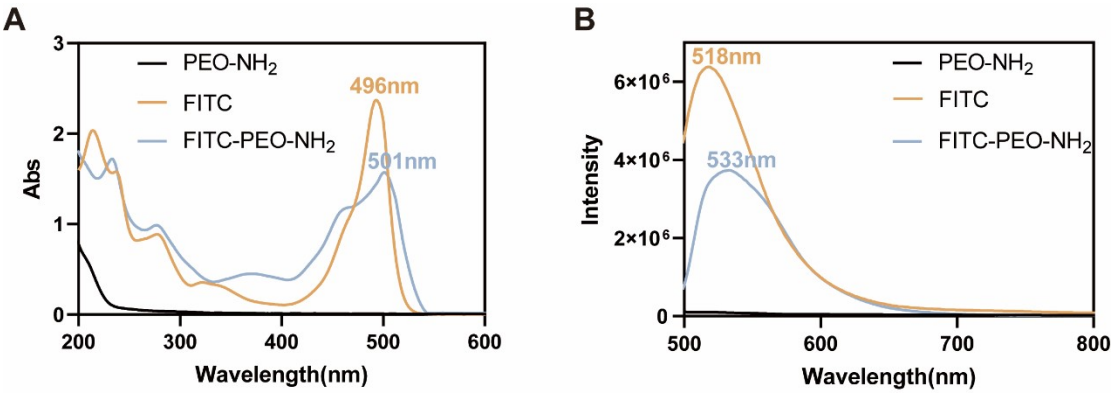


Fig. S11. A), UV-vis spectrum of FITC-PEO-NH_2. B), Fluorescence spectrum of FITC-PEO-NH_2.

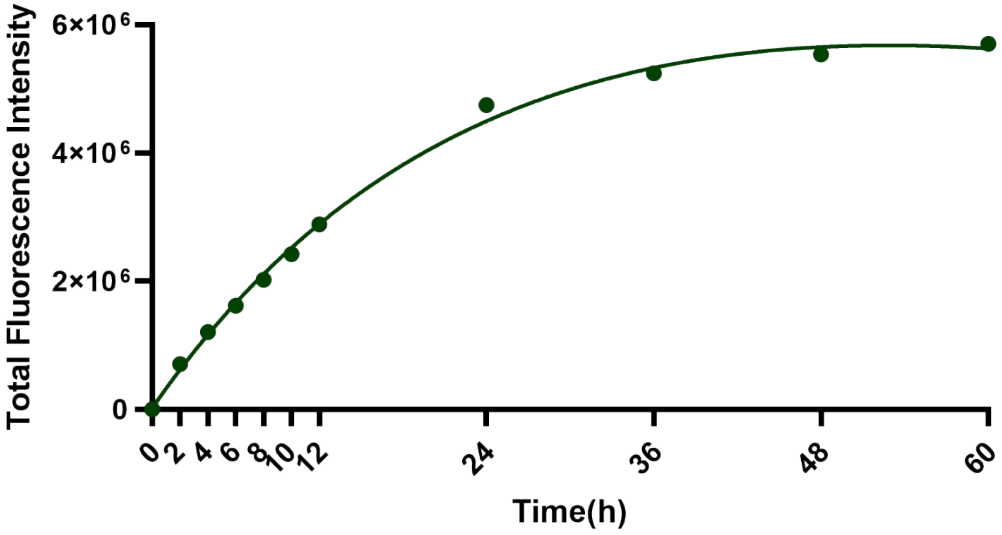


Fig. S12. After mixing GelMA with FITC-PEO-NH_2 solution to form a gel, the fluorescence intensity of PBS was measured after soaking in PBS.

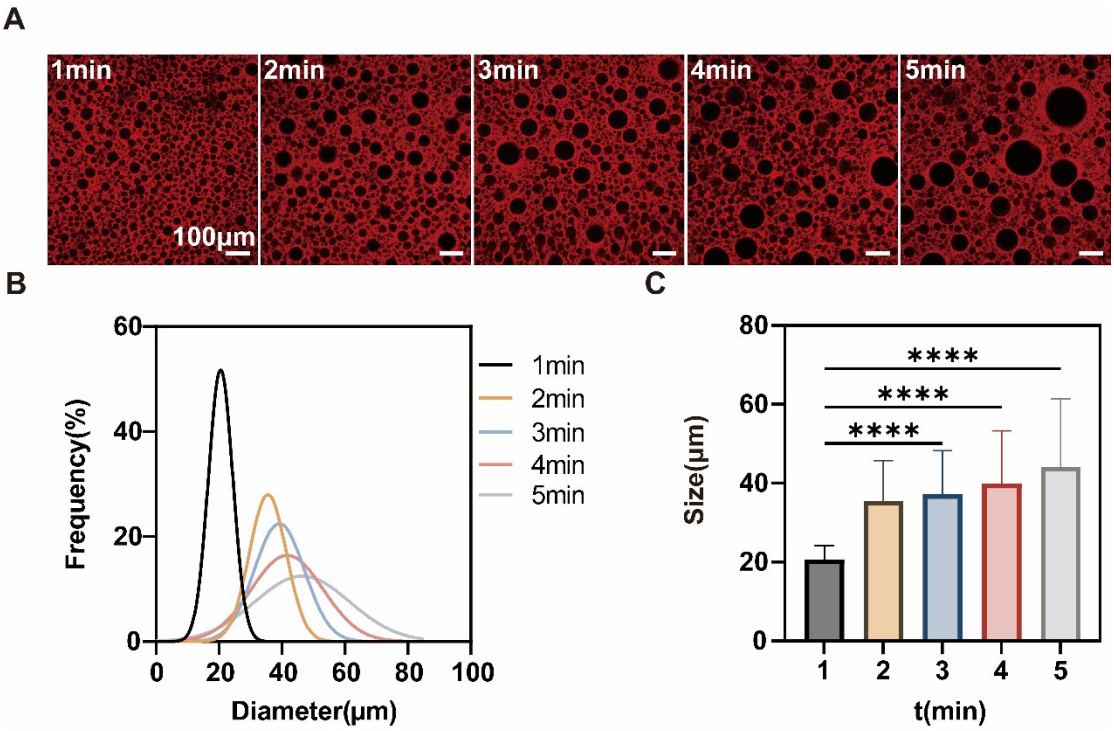


Fig. S13. A), CLSM observation of GelMA macroporous hydrogels with standing times of 1-5 minutes: a), 1 minute, b), 2 minutes, c), 3 minutes, d), 4 minutes, e), 5 minutes. Scale bar: 100 μm. B), Pore size distribution of GelMA macroporous hydrogels with standing times of 1-5 minutes. C), Average pore size of GelMA macroporous hydrogels with standing times of 1-5 minutes. (mean ± SD, $n = 3$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$)

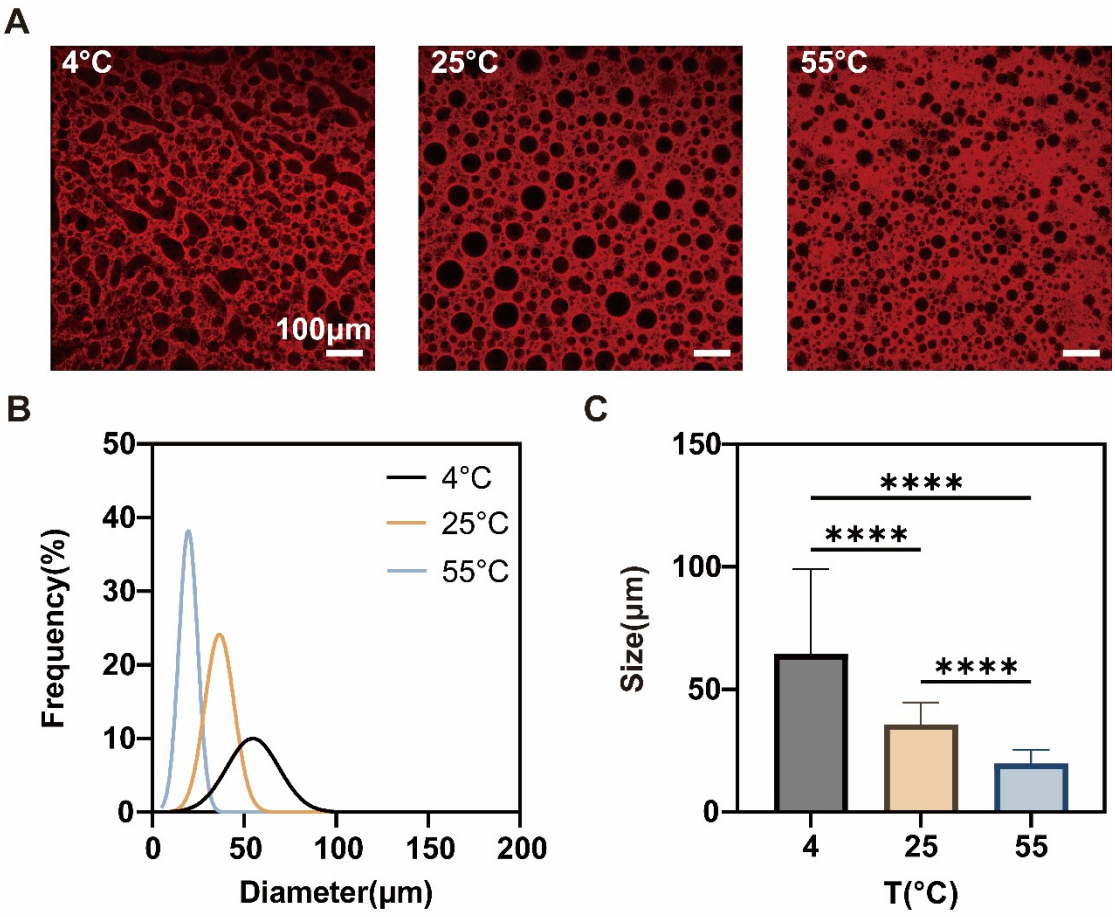


Fig. S14. A), CLSM observation of GelMA macroporous hydrogels at different ambient temperature: a), 4 °C, b), 25 °C, c), 55 °C. Scale bar: 100 μm. B), Pore size distribution of GelMA macroporous hydrogels at different ambient temperature. C), Average pore size of GelMA macroporous hydrogels at different ambient temperature. (mean ± SD, $n = 3$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$)

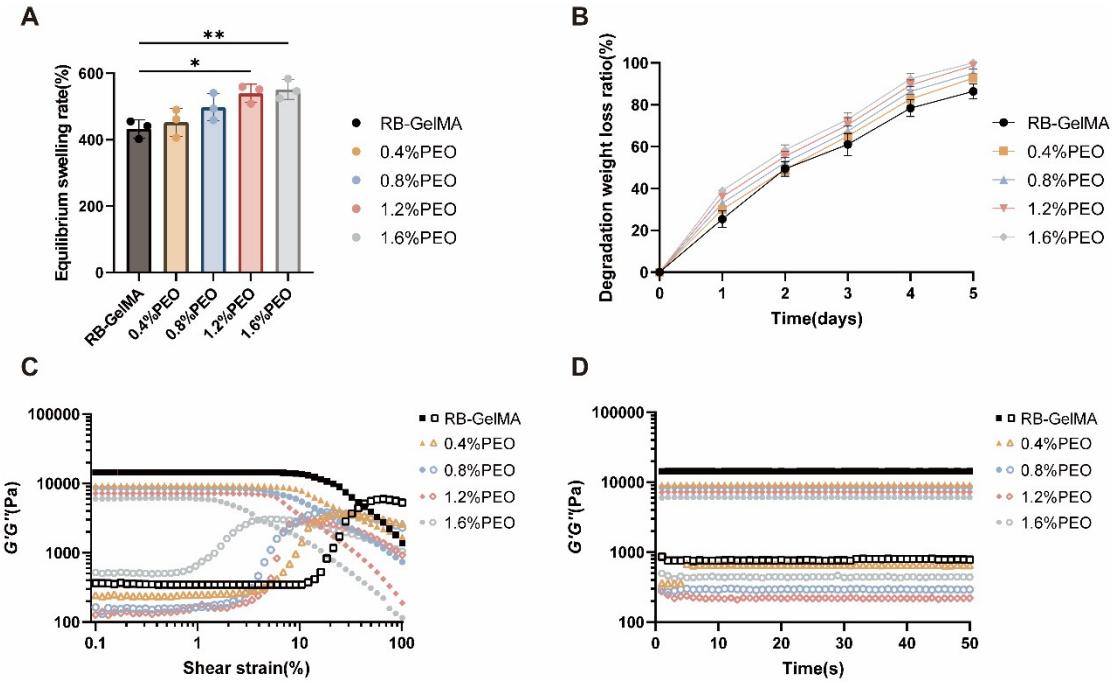


Fig. S15. Performance characterization of GelMA hydrogels and GelMA macroporous hydrogels with different pore sizes. A), Equilibrium swelling rate. B), Degradation performance in type I collagenase solution (20 U/mL in PBS) at 37 °C. C), Time sweep of dynamic rheology study. D), Oscillatory-amplitude sweep of dynamic rheology study. (mean ± SD, $n = 3$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$)

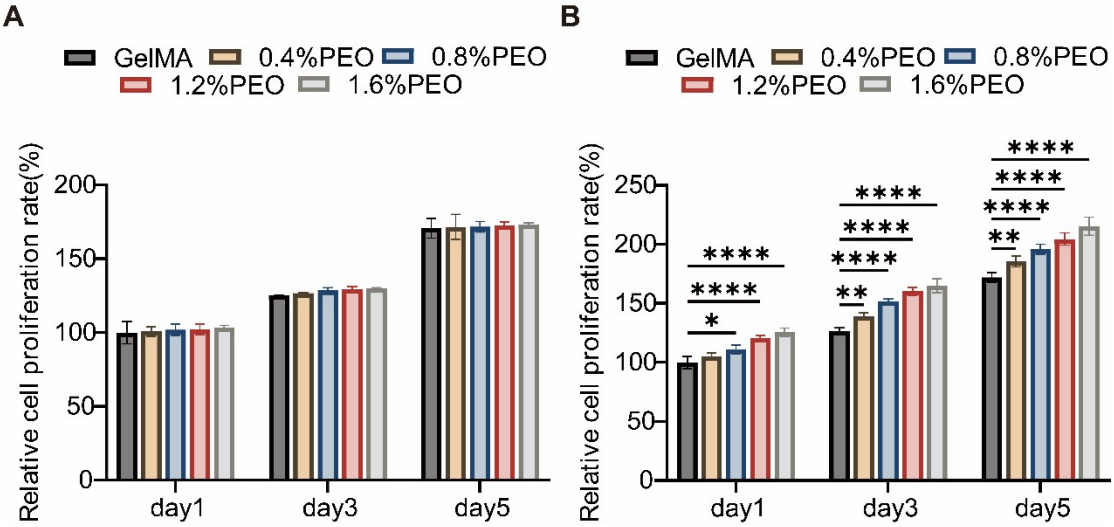


Fig. S16. Cell proliferation rates of GelMA hydrogels and GelMA macroporous hydrogels. A), Proliferation rate of BMSCs in the extract solution. B), Relative proliferation rate of BMSCs in two-dimensional culture. (mean ± SD, $n = 3$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$)

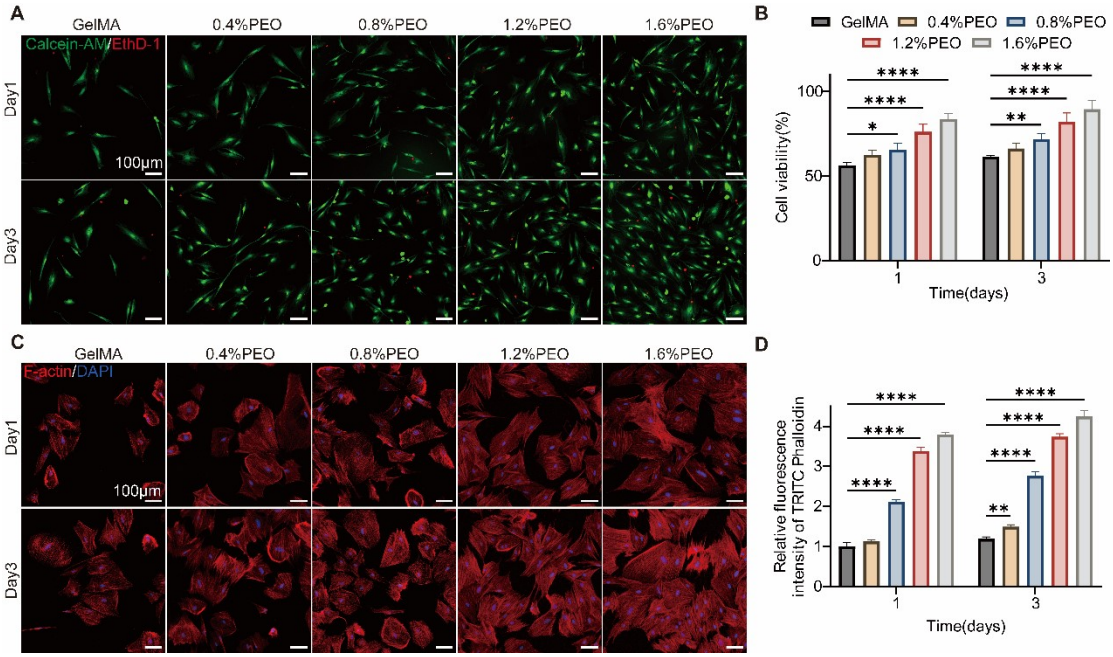


Fig. S17. Observation on the cell viability and morphology of BMSCs in two-dimensional culture using different pore diameters of GelMA hydrogels and GelMA macroporous hydrogels. A), Live/dead staining on day 1 and day 3. Scale bar: 100 μm. B), Cell viability. C), Fluorescent staining of the cytoskeleton of BMSCs on days 1 and 3. Scale bar: 100 μm. D), Quantitative analysis of the relative fluorescence intensity of F-actin based on inset C. (mean ± SD, $n = 3$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$)

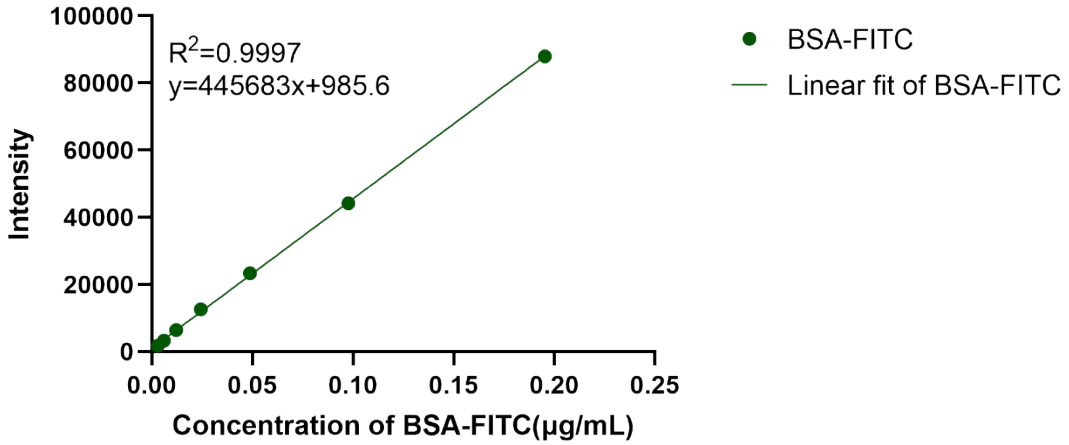


Fig. S18. Standard curve equation for BSA-FITC.

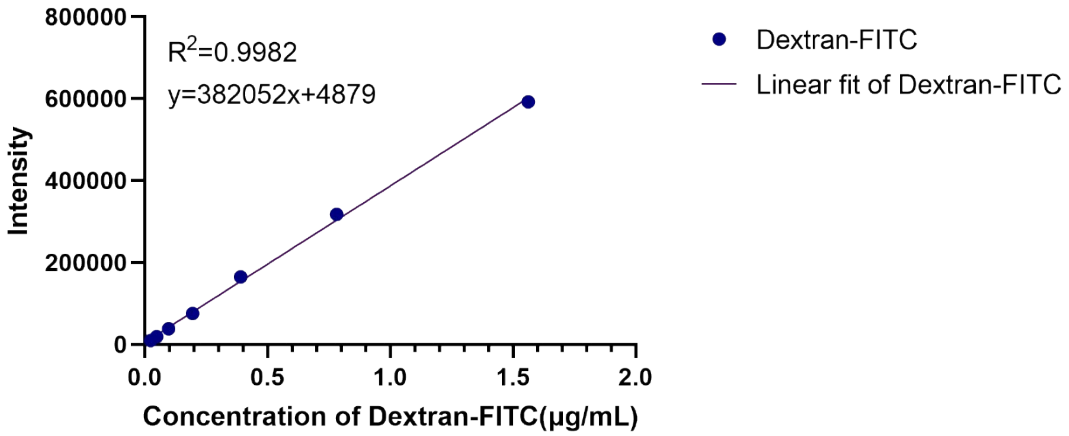


Fig. S19. Standard curve equation for Dextran-FITC.

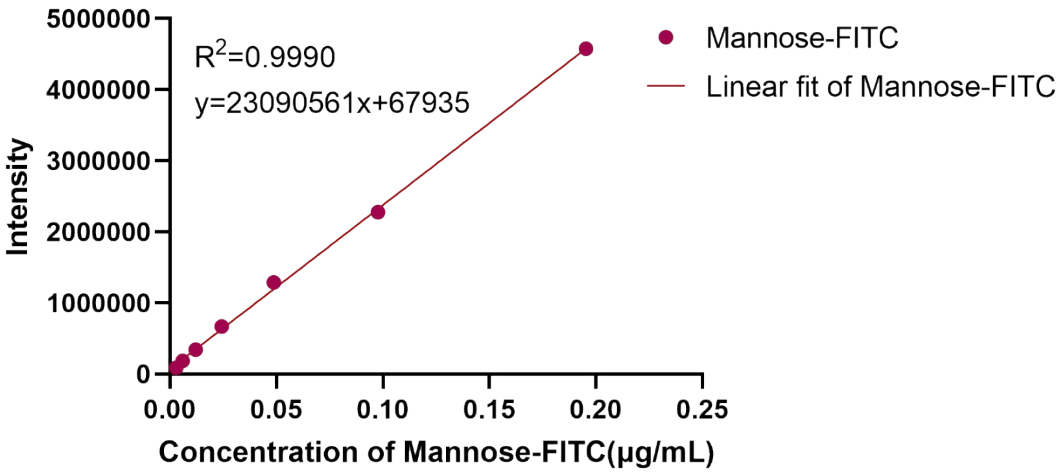


Fig. S20. Standard curve equation for Mannose-FITC.

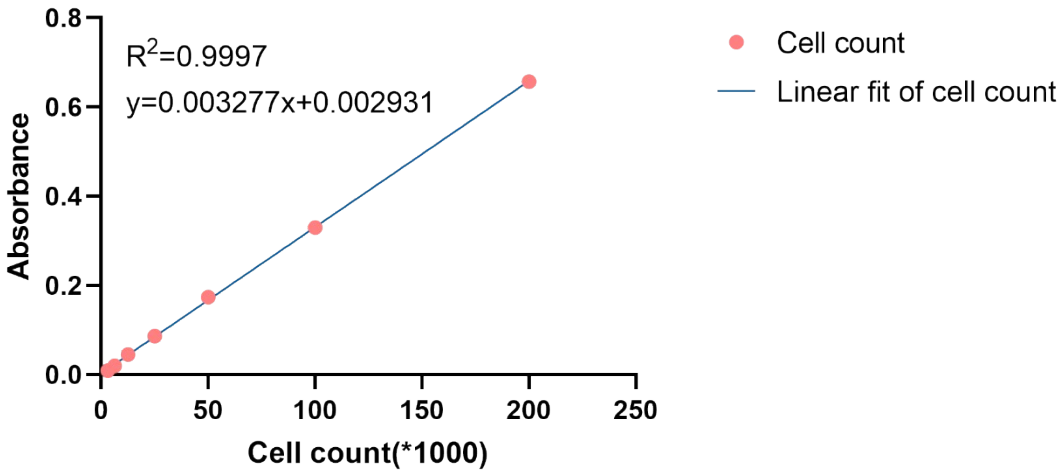


Fig. S21. Standard curve equation for cell count-absorbance.

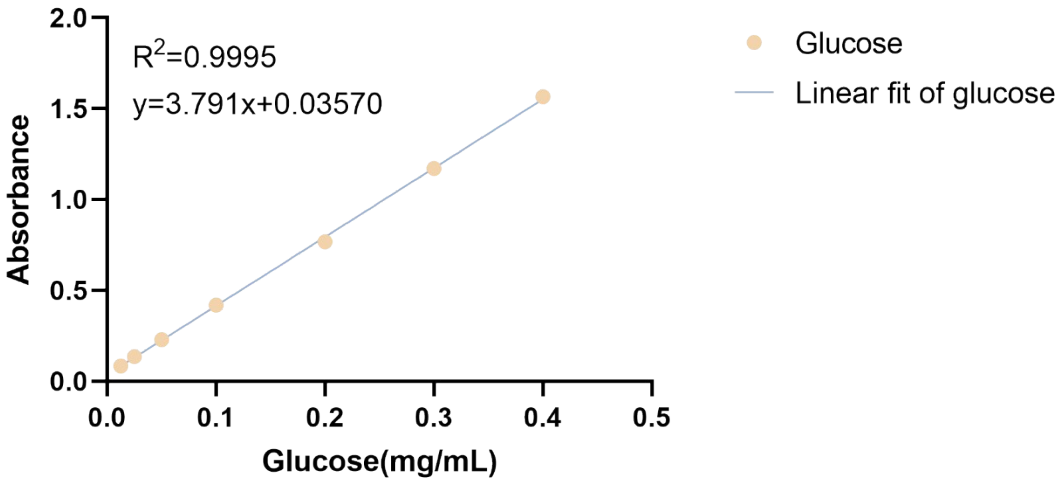


Fig. S22. Standard curve equation for glucose concentration-absorbance.

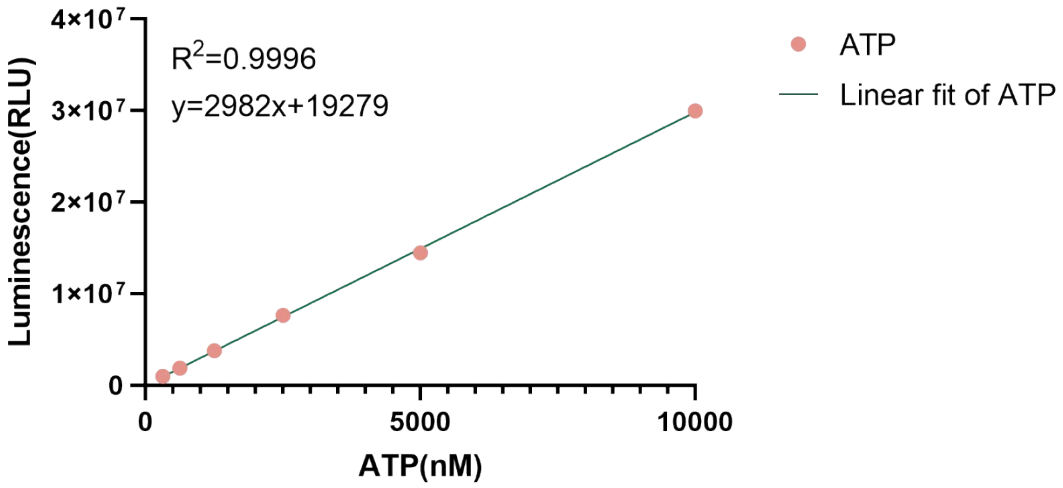


Fig. S23. Standard curve equation for ATP concentration-luminescence.

Supplementary References

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