SUPPLEMENTARY INFORMATION

Biodegradable nanofibrous temperature-responsive gelling microspheres for heart regeneration

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Section S1. PLLA-PEG-PNIPAm tri-block copolymer synthesis

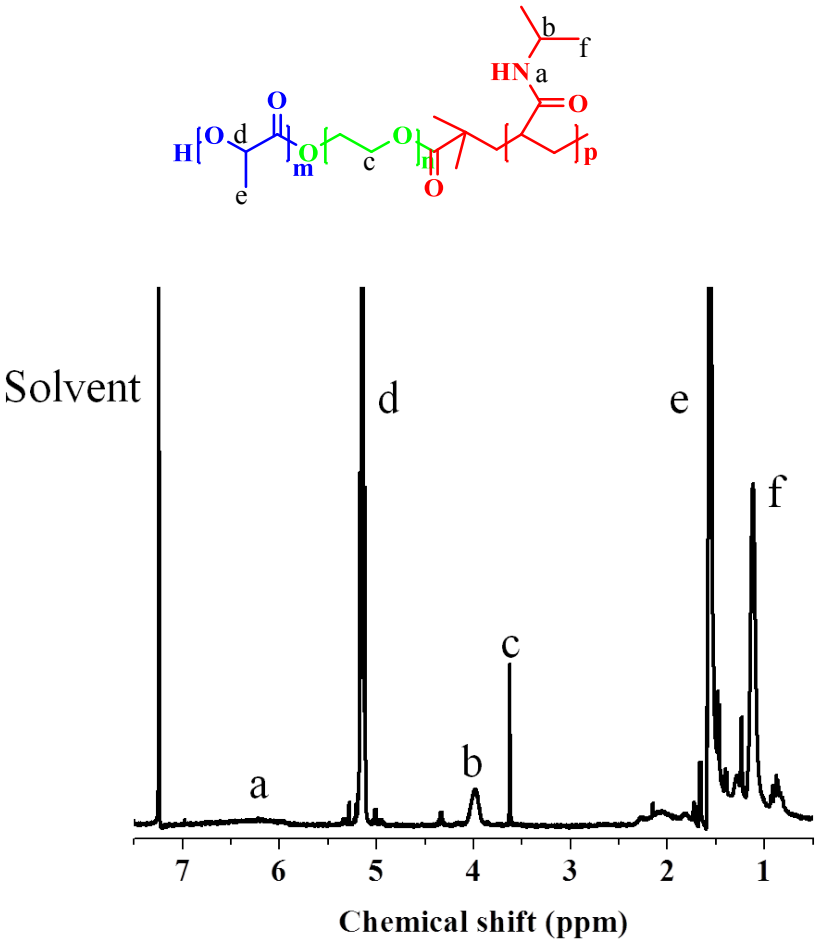


Figure S1. ^{1}H NMR spectrum of PLLA-PEG-PNIPAm. The resonance bands observed in the regions of 5.2 (d) and 1.6 (e) ppm are attributed to methine protons in the backbone and the pendent methyl protons of PLLA, respectively. Band at 3.6 (c) ppm is attributed to methylene protons of PEG. Bands at 1.2 (f) ppm, 4.0 (b) ppm and 6.4 (a) ppm are attributed to methyl protons**,** pendent methine protons and amine protons of PNIPAm, respectively.

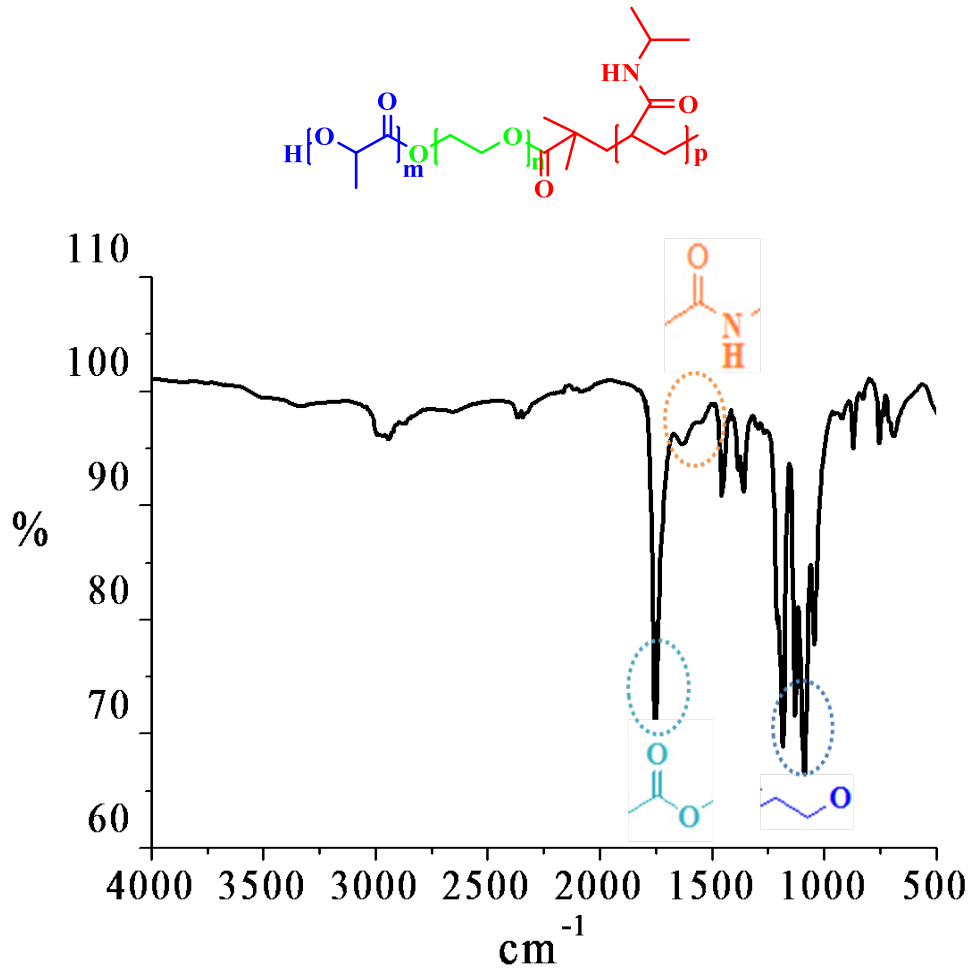


Figure S2. FTIR spectrum of PLLA-PEG-PNIPAm. The stretching vibration of the C=O (ester) groups of PLLA block appeared at 1750 cm^{-1}, the stretching vibration of the C-O groups of PEG block appeared at 1000-1250 cm^{-1}, and the stretching vibration of the C=O (amide) groups of PNIPAm block appeared at 1650 cm^{-1}.

Table S1. Molecular weight and molecular weight distribution[1] of polymers, and the microstructure and gelation property of corresponding microspheres.

Polymer	Mn	Mw	Mw/Mn	PEG Percentage (wt%)[2]	PNIPAm Percentage (wt%)[2]	PLLA Percentage (wt%)[2]	Microstructure of Microspheres	Free Standing Hydrogel[3]
PLLA-PEG$_{1000}$-PNIPAm	1904	2209	1.16	43	26	31	Smooth	Yes
PLLA-PEG$_{2000}$-PNIPAm[a]	6376	7286	1.14	24	60	16	Smooth	Yes
PLLA-PEG$_{4000}$-PNIPAm	4869	5192	1.07	57	21	22	Smooth	Yes
PLLA-PEG$_{1000}$-PNIPAm[e]	4200	4914	1.17	19	12	69	Platelet	Yes
PLLA-PEG$_{2000}$-PNIPAm[b]	8042	8726	1.09	19	48	33	Platelet	Yes
PLLA-PEG$_{2000}$-PNIPAm[d]	12584	15227	1.21	6	37	57	Platelet	Yes
PLLA-PEG$_{2000}$-PNIPAm	14775	22754	1.54	10	40	50	Platelet	Yes
PLLA-PEG$_{4000}$-PNIPAm	8157	13385	1.53	34	13	53	Platelet	Yes
PLLA-PEG$_{2000}$-PNIPAm[c]	**16665**	**23521**	**1.41**	**9**	**23**	**68**	**Nanofibrous[4]**	**Yes**
PLLA-PEG$_{4000}$-PNIPAm	**31008**	**48372**	**1.56**	**9**	**11**	**80**	**Nanofibrous**	**Yes**
PLLA-PEG$_{1000}$-PNIPAm[f]	**16256**	**22271**	**1.37**	**5**	**11**	**84**	**Nanofibrous**	**Yes**
PLLA-PEG$_{1000}$-PNIPAm[g]	21005	30247	1.44	4	8	88	Nanofibrous	No
PLLA-PEG$_{2000}$-PNIPAm	31020	44669	1.44	5	8	87	Nanofibrous	No
PLLA-PEG$_{1000}$-PNIPAm	7001	9101	1.30	12	7	81	Nanofibrous	No
PLLA-PEG$_{4000}$-PNIPAm	18553	26345	1.42	15	6	79	Nanofibrous	No
PLLA-PEG$_{4000}$-PNIPAm	35465	44331	1.25	8	3	89	Nanofibrous	No
PLLA-PEG$_{1000}$-PNIPAm	22654	31942	1.41	3	3	94	Nanofibrous	No
PLLA-PEG$_{2000}$-PNIPAm	45683	68524	1.50	3	9	88	Nanofibrous	No
PLLA-PEG$_{4000}$-PNIPAm	18553	26345	1.42	15	6	79	Nanofibrous	No
PLLA-PEG$_{4000}$-PNIPAm	35465	44331	1.25	8	3	89	Nanofibrous	No

1: Both the number-averaged (Mn) and weight-averaged (Mw) molecular weights (g/mol) of the copolymers were measured using GPC with THF as eluent and calculated using polystyrene standards.

2: PEG percentage (wt%)= $Mn_{PEG}/Mn_{copolymer}$; PNIPAm percentage (wt%)= $Mn_{PNIPAm}/Mn_{copolymer}$; PLLA percentage (wt%)= $Mn_{PLLA}/Mn_{copolymer}$;

3: If the microspheres can form hydrogel as shown in Fig. 4b at 37 ℃ at concentration from 5-10 w/v %, the microspheres were deemed to have the capability of forming free standing hydrogel.

4: If over 90% of the microspheres, observed under SEM microscope, have a typical nanofibrous structure, the PLLA-PEG-PNIPAm copolymers were deemed to have the capability of forming nanofibrous structure.

Table S2. Molecular weight and molecular weight distribution of PLLA-PEG-PNIPAm tri-block copolymers as a function of initiator and feed ratio

Polymer	Feed mass ratio	Reaction time (hour)	Mn[2]	Mw	Mw/Mn
HO-PEG_{1000}-Br (DI[1]1)	/	/	810	907	1.12
HO-PEG_{1000}-PNIPAm (MI[1]1a)	NIPAm/DI1=10/1	24	1302	1588	1.22
PLLA-PEG_{1000}-PNIPAm	L-lactide/MI1a=3/1	24	1904	2209	1.16
PLLA-PEG_{1000}-PNIPAm	L-lactide/MI1a=5/1	24	4053	4985	1.23
PLLA-PEG_{1000}-PNIPAm[e]	L-lactide/MI1a=5/1	24	4200	4914	1.17
PLLA-PEG_{1000}-PNIPAm	L-lactide/MI1a=10/1	24	7001	9101	1.30
PLLA-PEG_{1000}-PNIPAm	L-lactide/MI1a=15/1	24	22654	31942	1.41
HO-PEG_{1000}-PNIPAm (MI1b)	NIPAm/DI1=10/1	48	2524	3231	1.28
PLLA-PEG_{1000}-PNIPAm[f]	L-lactide/MI1b=10/1	24	16256	22271	1.37
PLLA-PEG_{1000}-PNIPAm	L-lactide/MI1b=15/1	24	21005	30247	1.44
HO-PEG_{2000}-Br (DI2)			1551	1722	1.11
HO-PEG_{2000}-PNIPAm (MI2a)	NIPAm/DI2=10/1	24	5371	6846	1.27
PLLA-PEG_{2000}-PNIPAm[a]	L-lactide/MI2a=3/1	24	6376	7286	1.14
PLLA-PEG_{2000}-PNIPAm[b]	L-lactide/MI2a=5/1	24	8042	8726	1.09
PLLA-PEG_{2000}-PNIPAm[d]	L-lactide/MI2a=10/1.5	24	12584	15227	1.21
PLLA-PEG_{2000}-PNIPAm[c]	L-lactide/MI2a=10/1.5	24	16665	23521	1.41
PLLA-PEG_{2000}-PNIPAm	L-lactide/MI2a=15/1	24	45683	68524	1.50
HO-PEG_{2000}-PNIPAm (MI2b)	NIPAm/DI2=10/1	48	7404	9181	1.24
PLLA-PEG_{2000}-PNIPAm	L-lactide/MI2b=10/1	24	14775	22754	1.54
HO-PEG_{2000}-PNIPAm (MI2c)	NIPAm/DI2=10/1	24	5583	6670	1.20
PLLA-PEG_{2000}-PNIPAm	L-lactide/MI2c=15/1	24	31019	45288	1.46
HO-PEG_{2000}-PNIPAm (MI2d)	NIPAm/DI2=10/1	10	4032	4717	1.17
PLLA-PEG_{2000}-PNIPAm	L-lactide/MI2d=15/1	24	31020	44669	1.44
HO-PEG_{4000}-Br (DI3)			2753	3221	1.17
HO-PEG_{4000}-PNIPAm (MI3a)	NIPAm/DI3=10/1	24	6253	7691	1.23
PLLA-PEG_{4000}-PNIPAm	L-lactide/MI3a=15/1	24	31008	48372	1.56
HO-PEG_{4000}-PNIPAm (MI3b)	NIPAm/DI3=10/1	10	3794	4400	1.16
PLLA-PEG_{4000}-PNIPAm	L-lactide/MI3b=3/1	24	4869	5192	1.07
PLLA-PEG_{4000}-PNIPAm	L-lactide/MI3b=5/1	24	8157	13385	1.53
PLLA-PEG_{4000}-PNIPAm	L-lactide/MI3b=10/1	24	18553	26345	1.42
PLLA-PEG_{4000}-PNIPAm	L-lactide/MI3b=15/1	24	35465	44331	1.25
PLLA-PEG_{2000}	L-lactide/DI2=10/1	24	14452	17487	1.21
PLLA-PNIPAm	/	/	14565	16021	1.10

1: DI = Double headed initiator; MI = Mono-head initiator

2: Measured by GPC and calculated using polystyrene as standards and THF as eluent. The data refers to the average molecular weight of polymer.

Table S3. Molecular weight and molecular weight distribution of synthesized PLLA as a function of HEMA/L-lactide feed ratio

Polymer	Feed molar ratio	Mn	Mw	Mw/Mn
HEMA-PLLA1.25	HEMA/ L-lactide =1.25/100	5521	6901	1.25
HEMA-PLLA2.5	HEMA/ L-lactide =2.5/100	4700	5263	1.11
HEMA-PLLA5	HEMA/ L-lactide =5/100	3803	4727	1.24

Section S2. Thermo-responsive properties of PEG-PNIPAm and PLLA-PEG-PNIPAm copolymers

PNIPAm is one of the most widely used thermoresponsive polymers, having a hydrophilic amide group and a hydrophobic isopropyl group. The linear PNIPAm chain undergoes a rapid transition from an extended-coil conformation to a shrunken conformation at its lower critical solution temperature (LCST) of around 32-34°C. Many previous studies have reported that copolymerization of NIPAm with other monomers can cause significant shift of the original LCST of PNIPAm homopolymer [1]. Because the thermally-induced phase transition behavior is crucial for the new polymer's *in situ* crosslinking and the intended biomedical applications, we use dynamic light scattering (DLS) to monitor the critical solution temperature of the copolymers in water with varying proportions of hydrophilic and hydrophobic blocks.

PEG-PNIPAm copolymers form micelles above the LCST. The copolymer was well suspended in water with an average hydrodynamic diameter (Dh) of about 10 nm at the temperature below 34°C (Fig. S3a). By increasing temperature, the stretched PNIPAm chains collapsed, and the water-soluble PEG blocks were extended to the outer surface, the core-shell micelles were formed. It is worth noting that Dh became a constant above the transition temperature region, indicating that the PNIPAm block was fully collapsed and the LCST of PEG-PNIPAm copolymer was approximately 34°C. PLLA-PEG-PNIPAm^c copolymers formed micelles at all temperatures with PLLA block in the core and PEG-PNIPAm block in the shell. The Dh of the polymeric micelles remained constant at about 950 nm at the temperature below 32 °C (Fig. S3b). By increasing temperature, the stretched PNIPAm chains collapsed, and the Dh of the polymeric micelles shrank to 600 nm. As marked by the sudden change in the particle size caused by conformational transition, the LCST of PLLA-PEG-PNIPAm^c (See composition in Table 1) was about 32 °C.

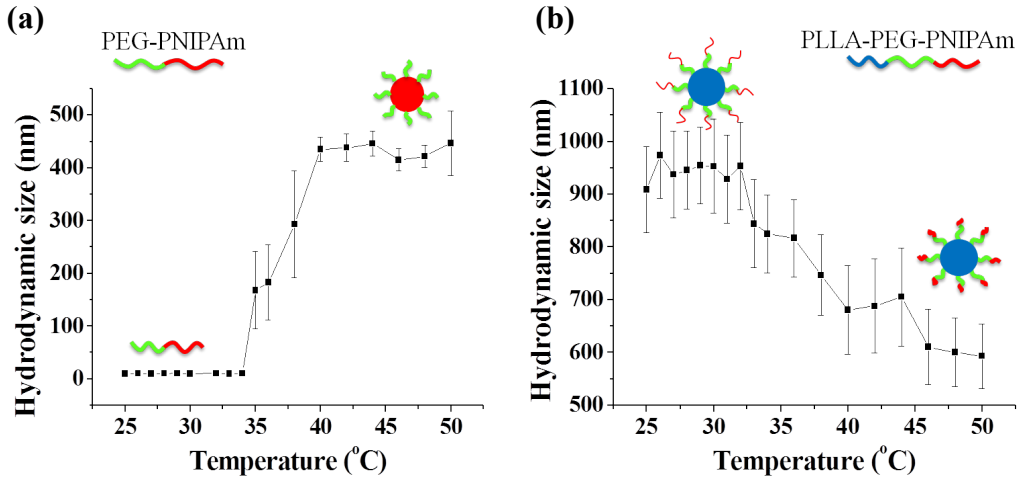


Figure S3. The hydrodynamic diameter of PEG-PNIPAm or PLLA-PEG-PNIPAm changing with temperature. (a). 0.1 wt% PEG-PNIPAm, (b) 0.01 wt% PLLA-PEG-PNIPAm^c in water as temperature ramps from 25 to 50°C

Section S3. NF-GMS fabrication and structure

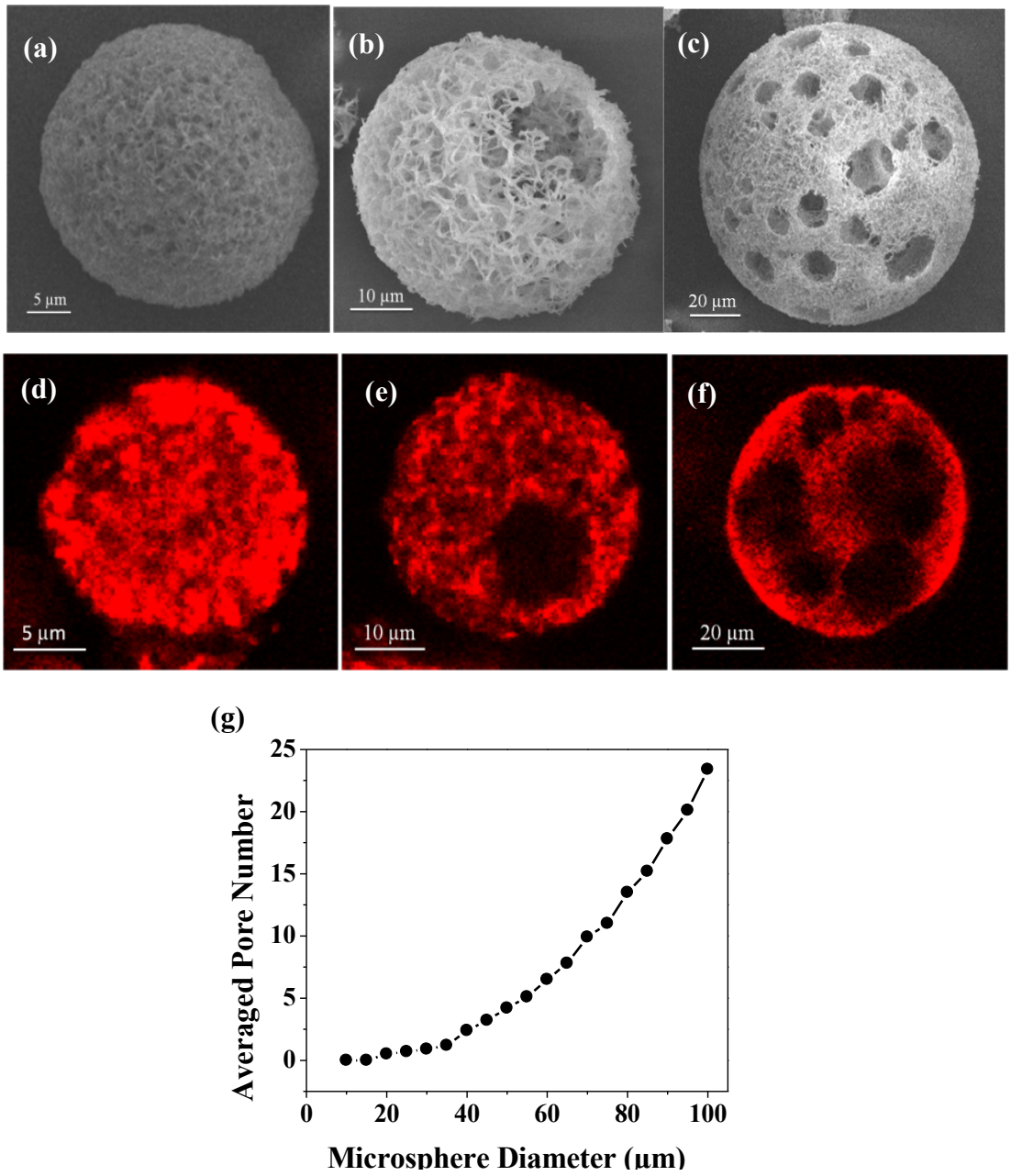
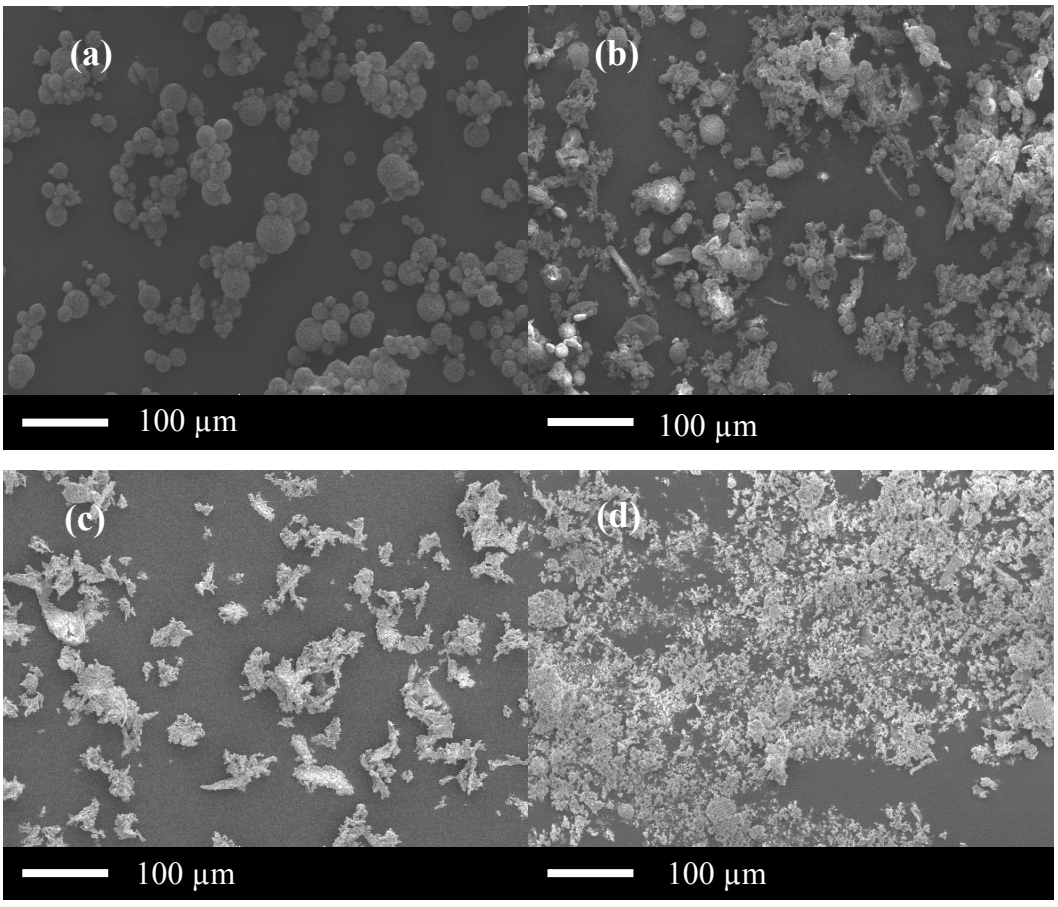


Figure S4. SEM micrographs and 2D cross-sectional fluorescence micrographs of nanofibrous microspheres fabricated from PLLA-PEG-PNIPAm^{c} with no open hole (a & d), one open hole (b & e) and multiple open holes (c & f). (g) Relationship of averaged pore number on one side of the microspheres with the diameter of the microspheres, 100 microspheres for each diameter were examined under SEM and the pore number was manually counted.

Section S4. NF-GMS in vitro degradation

The largest component in our triblock copolymer is PLLA, which is biodegradable through the hydrolysis of the ester bonds. The PEG and PNIPAm blocks are short in the triblock copolymers that can form nanofibers and hydrogels upon temperature change. Such relatively lower molecular weight PTG and PNIPAm can be excreted from the body after the PLLA block degradation. The in vitro degradation of PLLA-PEG-PNIPAm^c NF-GMS was examined using SEM and weight loss measurement. Prior to incubation in PBS, the NF-GMS had a typical spherical shape with open holes (Fig. S5). After 2 weeks of incubation, most of the NF-GMS still had a spherical shape, while some NF-GMS deformed. After 5 weeks of incubation, more NF-GMS deformed or disintegrated. After 8 weeks of incubation, the NF-GMS disintegrated entirely into small pieces. As shown in Fig. S6, while the pure PLLA NF-MS (30-60 μm, Mn = ~5521) lost only about 11% of the weight after 15 weeks of incubation in PBS. The PLLA-PEG-PNIPAm^c NF-GMS with diameters in the range of 30-60 μm and 60-90 μm lost more than 74% and 62% of their weights, respectively. The introduction of hydrophilic PEG and PNIPAm blocks into PLLA greatly likely enhanced the hydrophilicity and accelerated the degradation compared to PLLA-based NF microspheres.



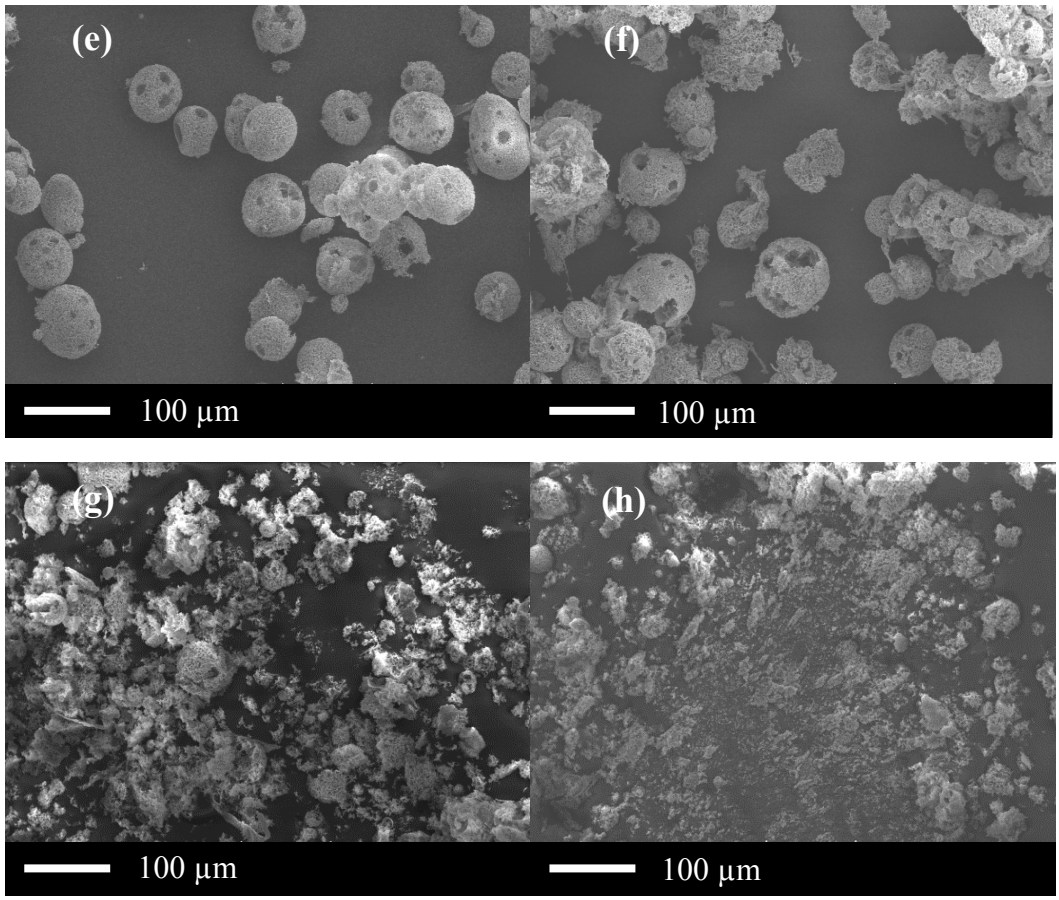


Figure S5. SEM micrographs of degrading PLLA-PEG-PNIPAM^c NF-GMS in PBS (a-d: 30-60 µm; e-h: 60-90 µm) for various time periods: (a, e) Prior to incubation in PBS, the NF-GMS had a typical spherical shape. (b, f) After 2 weeks of incubation, most of the NF-GMS still had a spherical shape, while some NF-GMS deformed. (c, g) After 5 weeks of incubation, more NF-GMS deformed or disintegrated. (d, h) After 8 weeks of incubation, the NF-GMS disintegrated entirely into small pieces.

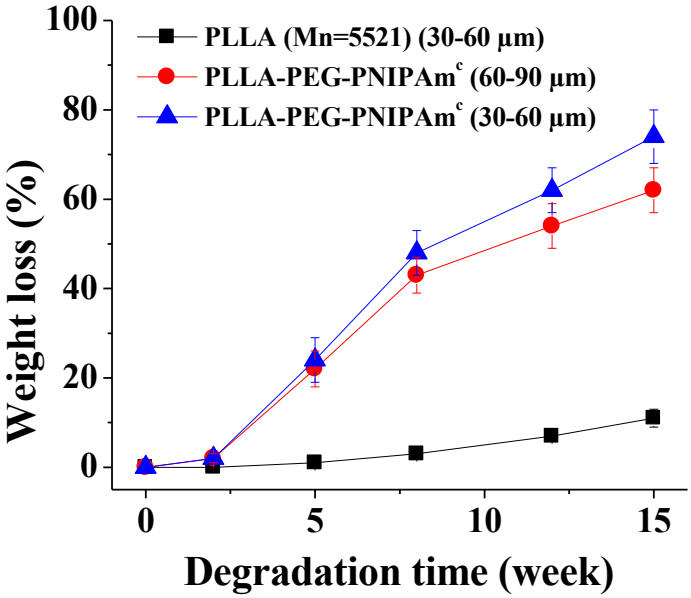


Figure S6. Weight loss of microspheres increased with incubation time in phosphate buffered saline (PBS) at 37°C. After 15 weeks of incubation in PBS, the PLLA-PEG-PNIPAm^c NF-GMS (30-60 µm) lost more than 74% of the weight, the PLLA-PEG-PNIPAm^c NF-GMS (60-90 µm) lost more than 62% of the weight, the pure PLLA nanofibrous microspheres (30-60 µm, Mn = 5521) lost only about 11% of their weights.

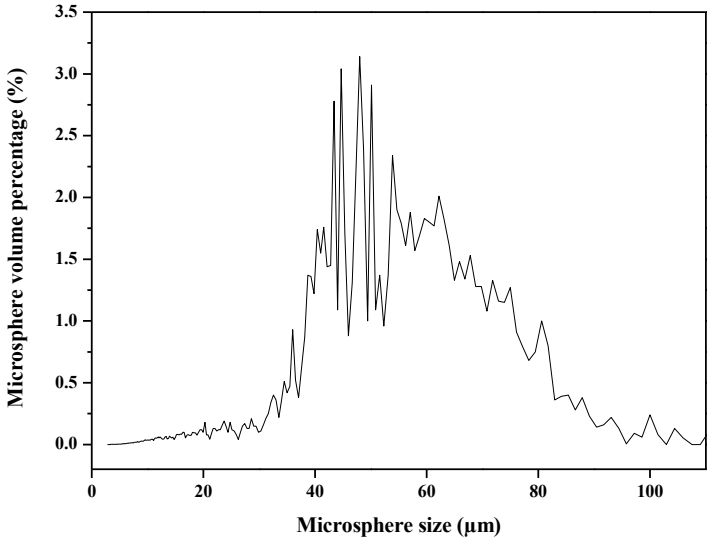


Figure S7. The size distribution of PLLA-PEG$_{2000}$-PNIPAm hollow microspheres (60-90 µm) collected from sieves with openings of 60 µm and 90 µm. More than 80 % microspheres fall in the rage of 40 µm to 90 µm with an average mean size of 54 µm, which is in good agreement with the SEM result.

Section S5. Viscoelastic mechanical properties of NF-GMS hydrogels

Rheological measurements were carried out to measure the sol-gel transition temperature and viscoelastic properties of NF-GMS. The storage modulus (G') of 10 w/v % NF-GMS (60-90 µm, PLLA/PEG/PNIPAm=68/9/23 wt%) increased from lower than 10 Pa at 30℃ to 8000 Pa at 45℃, showing the sol-gel transition temperature to be at approximately 35℃ (Fig. S7a). A similar phenomenon was observed for 10 w/v % PLLA-PEG-PNIPAm^{f} NF-GMS (Fig. S7b, PLLA/PEG/PNIPAm=84/5/11 wt%) and 10 w/v % PLLA-PEG-PNIPAm^{g} NF-HMS (Fig. S7c, PLLA/PEG/PNIPAm=88/4/8 wt%) aqueous suspension.

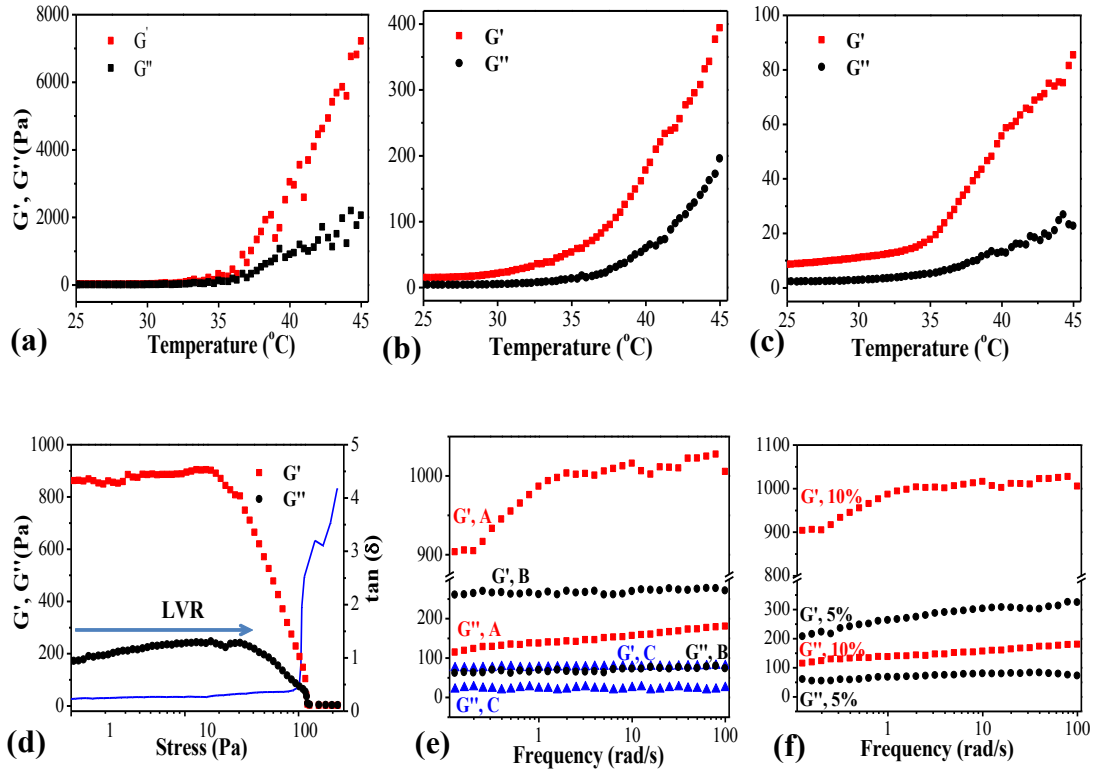


Figure S8. Temperature-responsive storage G' and loss G" modulus change of a) 10 w/v % PLLA-PEG-PNIPAm^{c} NF-GMS (**A**, 68/9/23 wt%), b) 10 w/v % PLLA-PEG-PNIPAm^{f} NF-GMS (**B**, 84/5/11wt%), c) 10 w/v % PLLA-PEG-PNIPAm NF-GMS (**C**, 88/4/8 wt%). d) Dynamic modulus at 37°C of 10 w/v % PLLA-PEG-PNIPAm^{c} (68/9/23 wt%) NF-GMS under increasing stress. Dynamic modulus at 37°C under increasing frequency of e) **A, B, C** NF-GMS with concentration of 10 w/v %, and f) PLLA-PEG-PNIPAm^{c} NF-GMS with concentration of 5 and 10 w/v %, a constant stress 0.1 Pa was applied.

To determine the strength and stability of NF-GMS, the stress sweep experiments were performed on the hydrogel of a 10 w/v % PLLA-PEG-PNIPAm^{c} NF-GMS (60-90 µm, 68/9/23 wt%) at 37°C.

A linear viscoelastic region (LVR) was observed at the stress level increasing from 0.1 to 20 Pa (Fig. S8d). When the applied stress was increased from 20 to 100 Pa, the storage modulus gradually decreased and the Tan(δ) (G"/G') increased. When the applied stress exceeded 100 Pa, the G' dropped dramatically from 145 to 2 Pa whilst the Tan(δ) increased from 0.5 to 2.5, indicating a yield stress level and the loss of mechanical integrity. Once the LVR was determined, frequency sweep experiments at a fix stress (0.1Pa) were performed at 37°C with rheological frequency increasing from 0.1 to 100 rad/s. The modulus showed frequency independence and G' was dominant over G" in the entire frequency range (Fig. S8e,f). To study the effect of PNIPAm percentage on the hydrogel strength, NF-GMS with various PNIPAm percentages were tested. With identical concentration (10 w/v %) and microsphere diameter range (60-90 μm), PLLA-PEG-PNIPAm^{c} (68/9/23 wt%) NF-GMS had the highest G' of 1000 Pa, PLLA-PEG-PNIPAm^{f} (84/5/11 wt%) NF-GMS had a G' of 260 Pa, and the PLLA-PEG-PNIPAm^{g} (88/4/8 wt%) NF-GMS had the lowest G' of 75 Pa. A similar phenomenon was observed for G", showing the positive correlation between the PNIPAm percentage (crosslinking density) and the mechanical properties of NF-GMS. To study the effect of NF-GMS concentration on the hydrogel mechanical properties, PLLA-PEG-PNIPAm^{c} (60-90 μm, 68/9/23 wt%) NF-GMS with two microsphere concentrations were tested. As the concentration decreased from 10 to 5 w/v %, G' and G" of the hydrogel decreased from 1000 to 300 Pa and 140 to 80 Pa, respectively (Fig. S8f), showing the correlation between the NF-GMS concentration and the mechanical properties of the NF-GMS hydrogel.

The results of temperature sweep, stress sweep and frequency sweep experiments consistently confirmed that aqueous suspension of PLLA-PEG-PNIPAm NF-GMS is a free-flowing, injectable liquid at room temperature, forms a mechanically useful physical hydrogel at 37℃, with a modulus in the order of magnitude of 10^1-10^3 Pa depending on both the PNIPAm percentage and NF-GMS concentration, which can be readily tuned for various biomedical applications.

Section S6. Effect of PLLA-PEG-PNIPAm composition on nanofibrous structure and gelation property of resulting microspheres

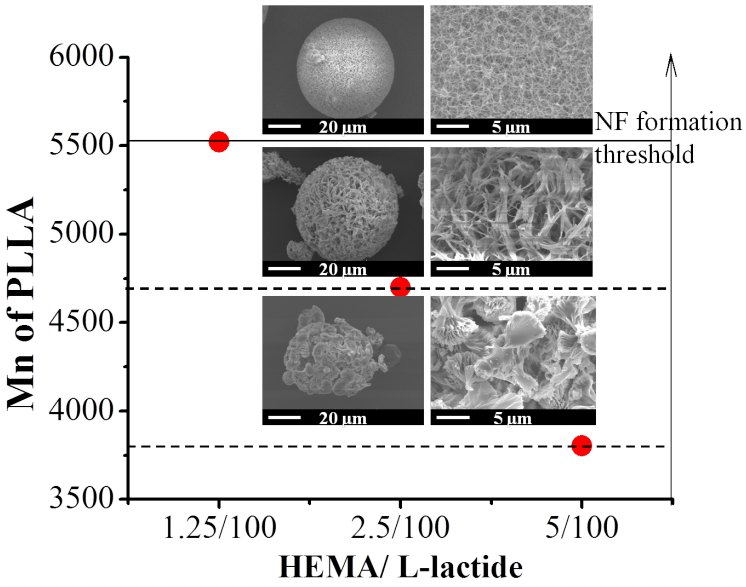
The effect of average molecular weight of PLLA homopolymer on the nanofibrous structure was first examined. PLLA homopolymers were synthesized via ROP of L-lactide using HEMA as the initiator catalyzed by $Sn(Oct)_2$, the average molecular weight was controlled by varying the initiator-to-monomer molar ratio (Table S2). At all Mn, PLLA homopolymers can be fabricated into microspheres (Fig. S9a). When the Mn of PLLA was ~3803, microspheres without the typical nanofibrous feature were fabricated. When the Mn of PLLA increased to ~4700, microspheres with a nanofibrous structure were fabricated. However, some of the fibers were stuck together, which led to the increase of the average fiber diameter. Microspheres with a typical nanofibrous structure were fabricated as the Mn increased to above about 5521. These results showed that the molecular weight has a critical effect on the PLLA microsphere structure.
For PLLA-PEG-PNIPAm copolymers, the addition of PEG and PNIPAm reduces the chain

regularity and the crystallinity of PLLA blocks. Therefore, the weight percentage of PLLA chains in the copolymers likely also affect the microsphere structure. When the weight percentage of PLLA block in the copolymers was in the range of ~16-31 wt%, microspheres with a smooth surface were fabricated (Fig. S9b and Table 1). When the weight percentage of PLLA in the copolymers was in the range of ~31-67 wt%, microspheres with a platelet-like structure were fabricated. And only when the weight percentage of PLLA in the copolymer was higher than ~68 wt%, microspheres with a typical nanofibrous structure were fabricated. AS specific examples, around 90% of PLLA-PEG_{2000}-PNIPAm^{c} microspheres (68 wt% PLLA) had a typical nanofiber structure, but only around 50% of PLLA-PEG_{2000}-PNIPAm^{d} microspheres (57 wt% PLLA) had a nanofiber structure (50% platelet-like structure). In this work, when 90% or higher microspheres in a batch exhibit the nanofibrous structure, the microspheres are defined to have a uniform nanofibrous structure.

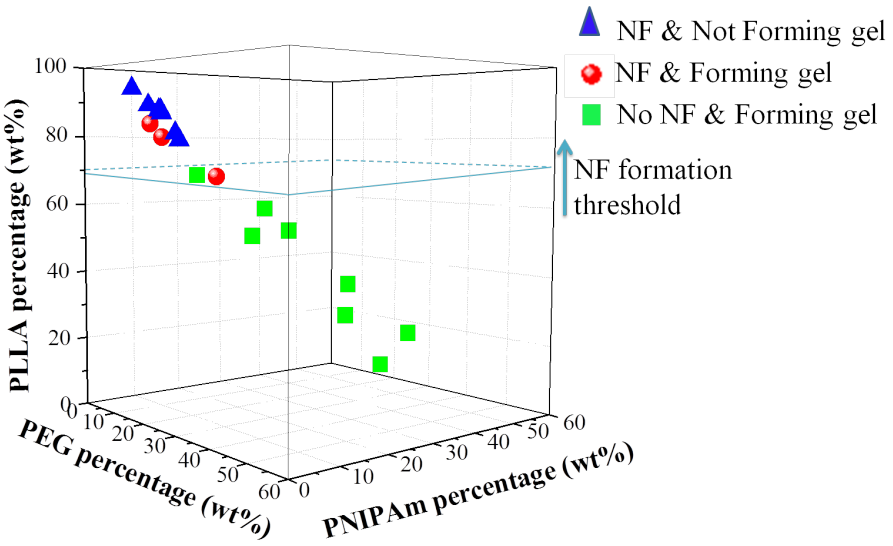
Thus, to fabricate PLLA-PEG-PNIPAm microspheres with a nanofibrous structure, two threshold requirements should be met simultaneously: Mn of the PLLA block should be higher than about 5521 and weight percentage of PLLA block in the copolymers should be higher than about 68 wt%. For example, although the Mn of PLLA block in PLLA-PEG-PNIPAm^{d} copolymer was ~7213 (Table 1), the weight percentage of PLLA was only ~57 wt %, which was below ~68 wt%. The microspheres made from PLLA-PEG-PNIPAm^{d} copolymer showed a platelet-like structure. For the PLLA-PEG-PNIPAm^{e} (Table 1), although the weight percentage of PLLA was ~69 wt%, the Mn of PLLA block was ~2898, which was far below ~5521. The microspheres with a platelet-like structure were made from PLLA-PEG-PNIPAm^{e} copolymer.

Similarly, there are two threshold requirements that should be met simultaneously to allow PLLA-PEG-PNIPAm microspheres to form a hydrogel: the PEG percentage should be about 5 wt% or higher and the PNIPAm percentage should be about 11 wt% or higher. A free-standing hydrogel was achieved as the weight percentage of PEG in the copolymer was in the range of ~5-28 wt% (Fig. S9c and Table 1). When the weight percentage of PEG block in the copolymer was less than about 5 wt%, precipitation happened due to the hydrophobic nature of the microspheres. This result demonstrated that 5 wt% of PEG block in the PLLA-PEG-PNIPAm copolymers was sufficient to work as the hydrophilic domain to bind water and prevent precipitation from occurring. Similarly, a free-standing hydrogel was achieved only when the weight percentage of PNIPAm block in the copolymers was about 11 wt% or higher. When the weight percentage of PNIPAm block in the copolymers was lower than 11 wt%, the hydrophobic interactions between PNIPAm blocks at body temperature was too weak to hold a strong enough physical network, and the PLLA-PEG-PNIPAm microspheres cannot form a free-standing hydrogel.

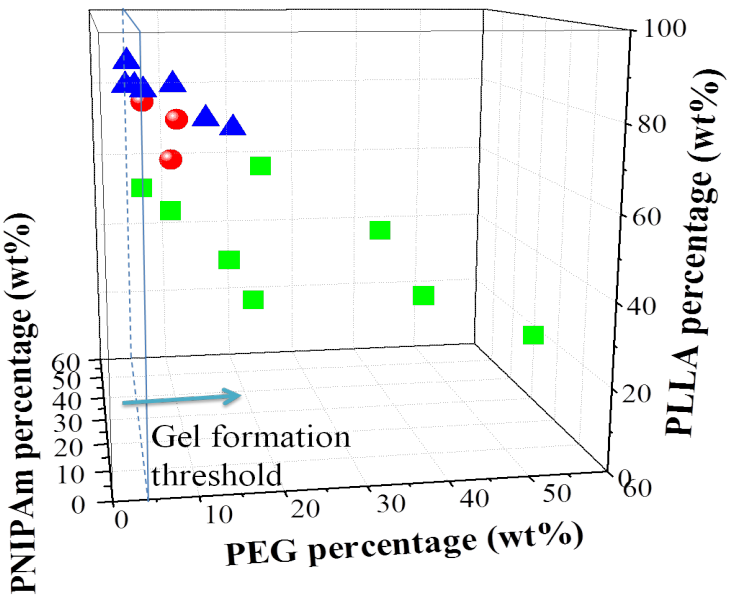
(a)


6000
5500
5000
4500
4000
3500
Mn of PLLA
1.25/100
2.5/100
5/100
HEMA/ L-lactide
20 μm
5 μm
NF formation
threshold

(b)


NF & Not Forming gel
NF & Forming gel
No NF & Forming gel
NF formation
threshold
PLLA percentage (wt%)
PEG percentage (wt%)
PNIPAm percentage (wt%)

(c)



(d)

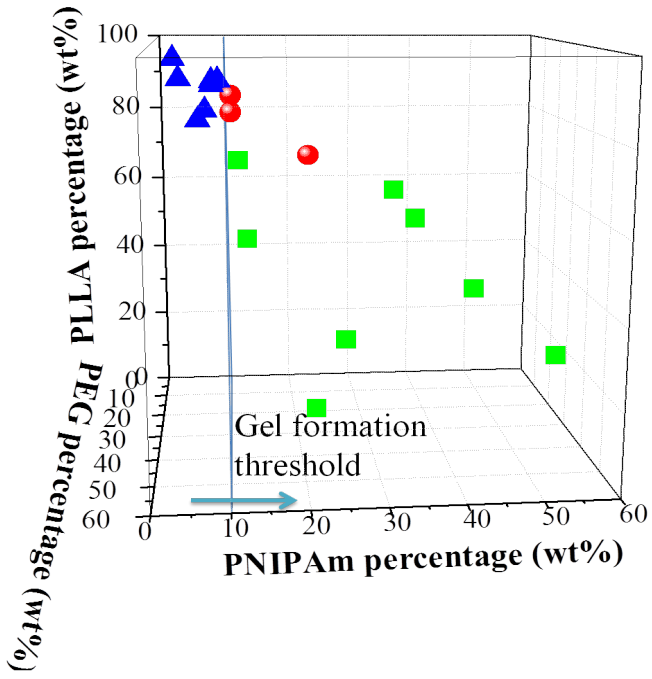


Figure S9. Structural requirements of PLLA-PEG-PNIPAm copolymer for nanofiber formation and hydrogel formation: NF formation threshold of (a) Mn of PLLA homopolymer should be higher than ~5521, (b) PLLA percentage in PLLA-PEG-PNIPAm copolymer should be about 68 wt% or higher. Gel formation threshold of (c) PEG percentage in PLLA-PEG-PNIPAm copolymer should be about 4 wt% or higher, (d) PNIPAm percentage in PLLA-PEG-PNIPAm copolymer should be about 11 wt% or higher.

Note: NF refers to nanofibrous structure; the Mn of PLLA block of all the copolymers tested in b,c,d is higher than ~5521.

Section S7. NF-GMS to carry cardiomyocytes for heart regeneration

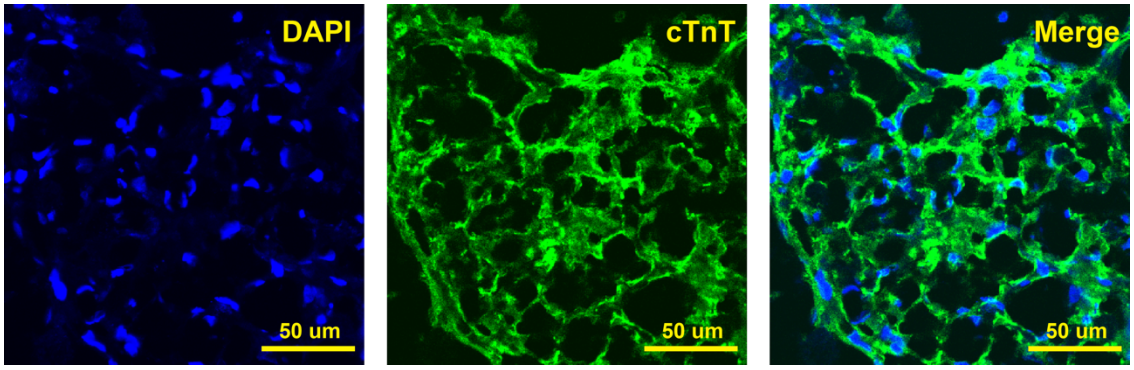


Figure S10. NF-GMS maintained cardiomyocyte (CM) phenotype in vitro. CMs were cultured with NF-GMS for 7 days and expressed the cardiac troponin T (cTnT, green), where DAPI (blue) stained for nuclei.

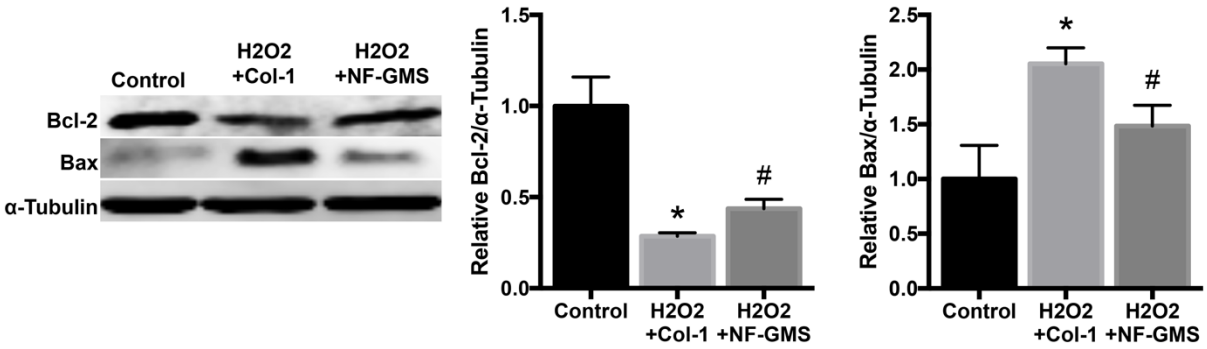


Figure S11. NF-GMS promoted cell survival compared to collagen I gel. CMs were co-cultured with Collagen-I (the major ECM in heart and particularly after MI injury) or NF-GMS with 50 μM H_2O_2 treatment for 4 h to mimic in vivo MI injury. NF-GMS significantly increased cell survival after H_2O_2 treatment compared with Collagen-I by Western blot with Bcl-2 (anti-apoptosis marker) and Bax (pro-apoptosis marker) antibodies.

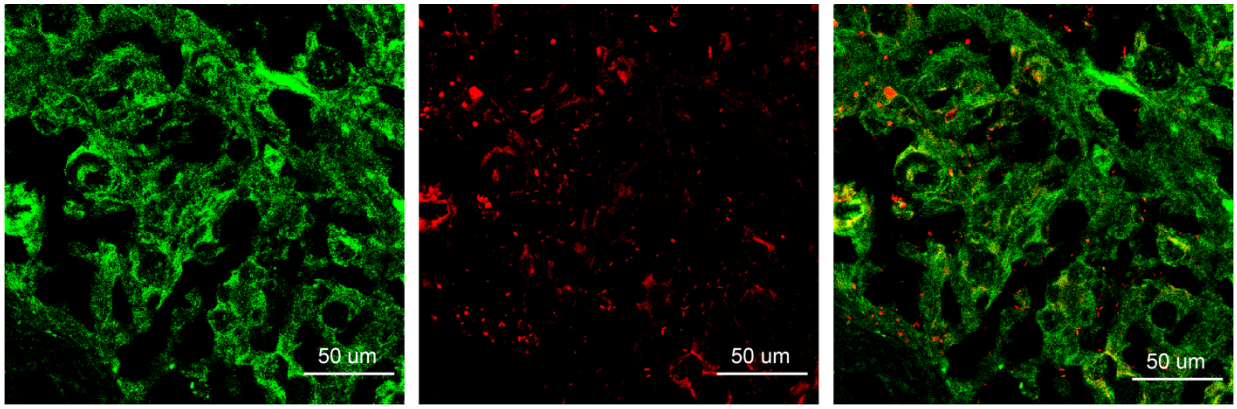


Figure S12. **Higher magnification images from the same specimen of the lower panel of Figure 5B.** CM engraftment was illustrated by human specific antigen (Green: Hu-mito) staining. Abundant gap junctions were formed (Red: Connexin 43). Left, hu-mito; Center, connexin 43; Right: merged.

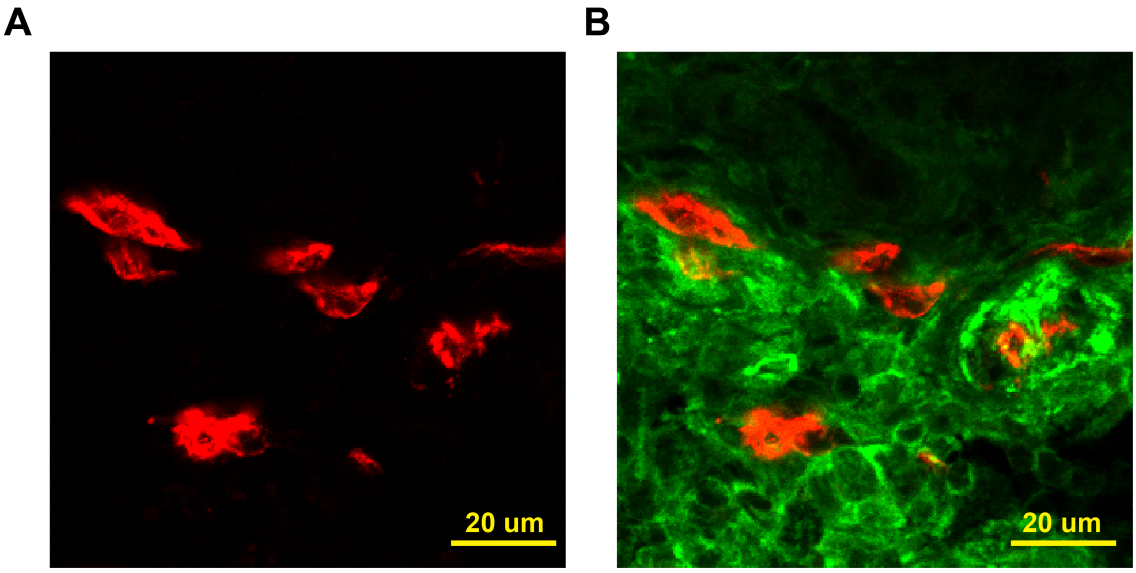


Figure S13. Stable vasculature in cell transplanted regions illustrated with immunostaining with antibodies against CD31 (red) and αSMA (green) in CM+NF-GMS group 4 weeks after transplantation. (A) CD31 only (red), (B) merged CD31 (red)/αSMA (green) double staining.

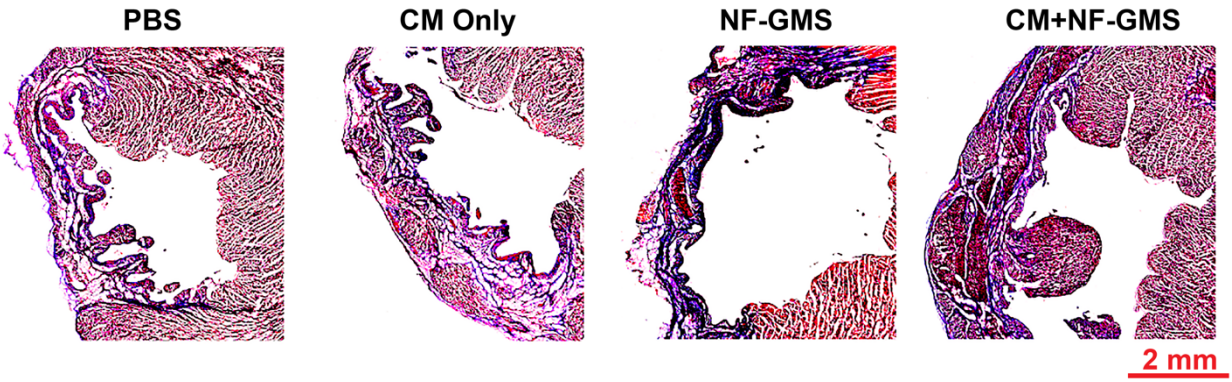


Figure S14. Higher magnification image of the lower panel of Figure 6A.

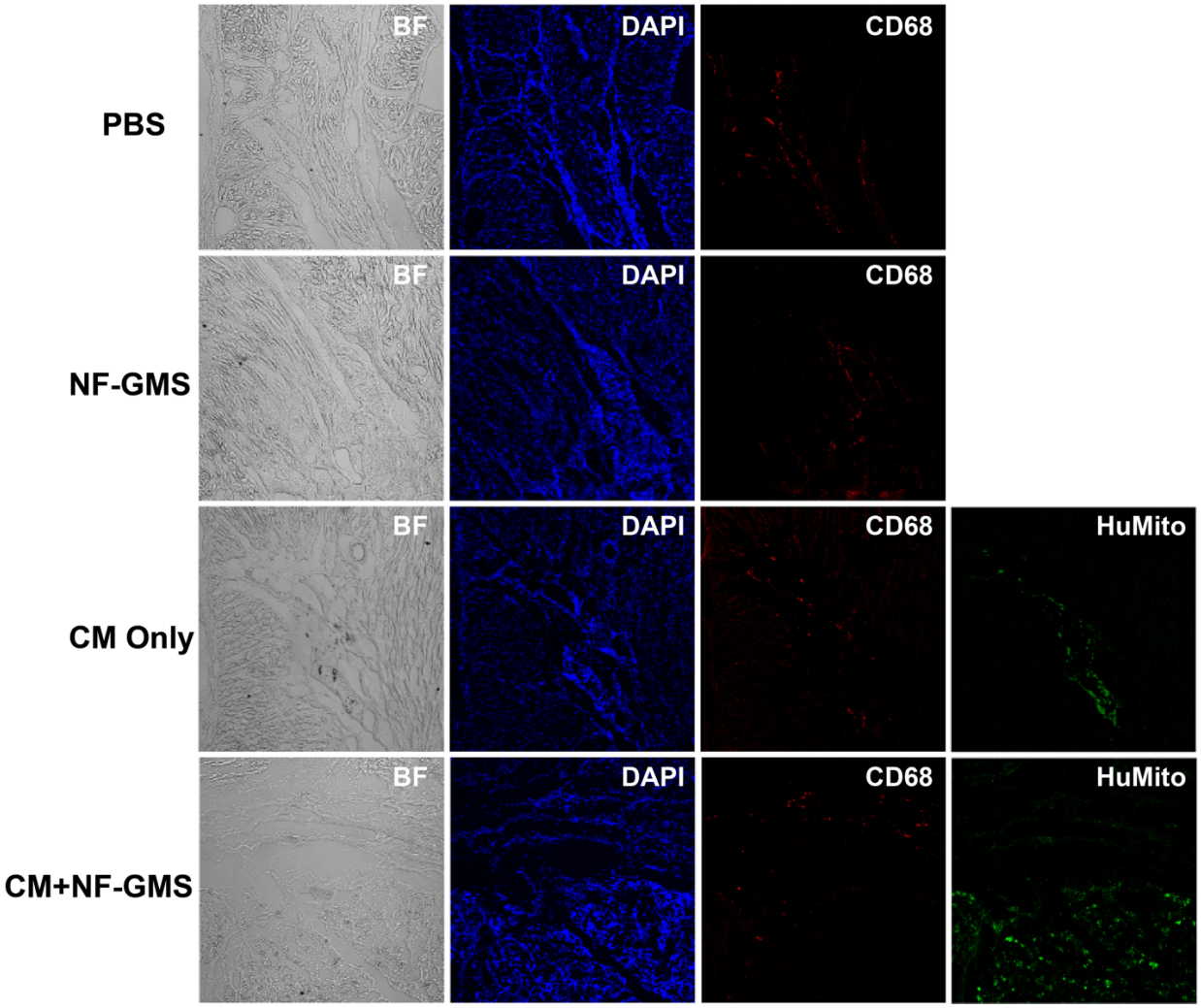


Figure S15. Examining immune cell infiltration. CD68 staining of MI heart 4 weeks after cell transplantation suggested that the modest immune cell infiltration (CD68+) was mainly caused

by MI injury and there were no significant differences between PBS, NF-GMS only, CM only, and CM+NF-GMS groups at this time.

Supplementary Materials and Methods

Materials: (3s)-cis-3,6-dimethyl-1,4-dioxane-2,5-dione (L-lactide, 98%), copper(I) chloride (CuCl, 99.999%), 2-bromoisobutyryl bromide (BIBB, 98%), glycerol (99%), 2-(2-bromoisobutyryloxy)ethyl methacrylate (95%), phosphate buffer saline (PBS, pH 7.4, 0.15 M, 138 mM NaCl, 2.7 mM KCl), 2-hydroxyethyl methacrylate (HEMA, 97%), poly(ethylene glycol) methacrylate (PEGMA, Mn=500), dichloromethane (99.8%), *N*-Isopropylacrylamide (NIPAm, 97%), fluorescein *o*-acrylate (95%), ethanol (absolute 200 proof), triethylamine (TEA, ≥99.5%), calcium hydroxide (95%), ethyl ether (absolute), methanol (99.5%), chloroform (≥99.5%), chloroform-d (100%, 99.96 atom % D), toluene (99.8%), tetrahydrofuran (THF, 99.9%) and stannous 2-ethylhexanoate ($Sn(Oct)_2$) were purchased from Sigma-Aldrich Inc. (St. Louis, MO). Nile blue acrylamide and acryloxyethyl thiocarbamoyl Rhodamine B were purchased from Polysciences Inc. (Warrington, PA). Tris[2-(dimethylamino)ethyl]amine (Me6TREN, 99%) was purchased from Alfa Aesar (Ward Hill, MA).

Synthesis of PLLA-PEG: PLLA-PEG was synthesized following the same procedure as the reaction 3 in Fig. 1b with the exception that OH-PEG-Br was used as the initiator for the ring-opening polymerization of L-lactide.

Synthesis of PLLA-PNIPAm: PLLA-PNIPAm was synthesized following the same procedure as reactions 2 and 3 with the exception that 2-(2-bromoisobutyryloxy)ethyl methacrylate was used as the double-headed initiator for ATRP of NIPAm and ROP of L-lactide.

Synthesis of PLLA homopolymer: PLLA homopolymers with varying number average molecular weights (Mn) were synthesized by the ring opening polymerization of L-lactide using HEMA as the initiator. The synthesized PLLA are abbreviated as HEMA-PLLA*X*, where *X* is the molar percentage of HEMA to L-lactide (*X*=5 means HEMA/L-lactide =5%). A typical procedure to synthesize HEMA-PLLA5 is as follows: L-lactide (40 mmol, 5.760 g), HEMA (2 mmol, 0.260 g) and $Sn(Oct)_2$ (0.4 mmol, 0.162 g) were mixed in a 50 mL round-bottom flask with stirring and nitrogen purging. The mixture was heated to 120°C ℃ under nitrogen protection for complete melting. The polymerization was carried out at 140°C for 2 h. The crude product was dissolved in 20 mL chloroform, precipitated in 100 mL cold methanol, and then vacuum dried.

Synthesis of florescence stained PLLA-PEG-PNIPAm block copolymers:
Synthesis of Br-PEG-PLLA copolymer: Dry THF (10 mL), L-lactide (139 mmol, 2 g), PEG macroinitiator (Mn=1551) (0.6 mmol, 1 g), and $Sn(Oct)_2$ (0.4 mmol, 0.162 g) were mixed in a 50 mL round-bottom flask with stirring and nitrogen purging. The mixture was heated to 80℃ under nitrogen protection for complete melting. The polymerization was carried out at 80℃ under nitrogen protection for 24 h. The crude product was dissolved in 20 mL THF, precipitated in 100 mL de-ionized (DI) water. The resulting mixture was then dialyzed (MW cut-off 3.5 kDa) against

de-ionized water for 3 days to remove unreacted PEG-macroinitiator. The mixture was then lyophilized for three days to give Br-PEG-PLLA copolymer.

Synthesis of fluorescein o-acrylate stained PLLA-PEG copolymer: Br-PEG-PLLA copolymer (1 g), Fluorescein o-acrylate (0.065 mmol, 0.025 g), and CuCl (0.170 mmol, 0.0168 g) were placed in a 250 mL round-bottom flask under nitrogen protection and sealed with rubber septum stoppers. Milli-Q water (20 mL) and Me_6TREN (0.174 mmol, 0.04 g) were placed in a Schlenk tube and purged with N_2 gas for 40 min. The solution was transferred to the round-bottom flask using a syringe under nitrogen protection. The reaction mixture was then stirred under nitrogen atmosphere for 24 h. The reaction was then stopped by opening the vessel to air. The reaction mixture was dialyzed (MW cut-off 3.5 kDa) against DI water for 3 days to remove unreacted Fluorescein o-acrylate. The mixture was then lyophilized for 3 days to give fluorescein o-acrylate stained PLLA-PEG copolymer.

Synthesis of Fluorescein o-acrylate (PEG) and acryloxyethyl thiocarbamoyl Rhodamine B (PNIPAm) stained PLLA-PEG-PNIPAm copolymer: Fluorescein o-acrylate stained PLLA-PEG copolymer (0.5 g), acryloxyethyl thiocarbamoyl Rhodamine B (0.007 mmol, 0.005 g), NIPAm (26.5 mmol, 3 g) and CuCl (0.170 mmol, 0.016.8 g) were placed in a 250 mL round-bottom flask under nitrogen protection and sealed with rubber septum stoppers. Milli-Q water (20 mL) and Me_6TREN (0.174 mmol, 0.04 g) were placed in a Schlenk tube and purged with N_2 gas for 40 min. The solution was transferred to the round-bottom flask using a syringe under nitrogen protection. The reaction mixture was then stirred under nitrogen atmosphere for 24 h. The reaction was then stopped by opening the vessel to air. The reaction mixture was dialyzed (MW cut-off 3.5 kDa) against DI water for 3 days to remove unreacted acryloxyethyl thiocarbamoyl Rhodamine B. The mixture was then lyophilized for 3 days to give Fluorescein o-acrylate and acryloxyethyl thiocarbamoyl Rhodamine B stained PLLA-PEG-PNIPAm copolymer.

Synthesis of Nile blue acrylamide stained PLLA polymer: HEMA-PLLA5 (1.4 g), Nile blue acrylamide (0.012 mmol, 0.005 g), and AIBN (0.06 mmol, 9.8 mg) were added into dioxane (10 mL) and stirred until dissolved. The polymerization was carried out at 70℃ for 24 h. After polymerization, the crude product was purified by repeated re-precipitations from chloroform to methanol for 3 times, and finally vacuum dried at 40℃ for 48 h to give Nile blue acrylamide stained PLLA polymer.

Fabrication of florescence stained nanofibrous microspheres.

PLLA-PEG-PNIPAm copolymer was dissolved in 20 mL THF at 60℃ with a concentration of 2.0% (wt/v) fluorescein o-acrylate and acryloxyethyl thiocarbamoyl Rhodamine B stained PLLA-PEG-PNIPAm copolymer (0.1 g) and Nile blue acrylamide stained PLLA polymer (0.05 g) were added and dissolved. Under rigorous mechanical stirring (speed 7, MAXIMA, Fisher Scientific), glycerol (60℃) with 3 times the volume of the PLLA-PEG-PNIPAm copolymer solution was gradually added into the PLLA-PEG-PNIPAm copolymer solution. Stirring was continued for 5 min

afterwards. The mixture was then quickly poured into liquid nitrogen. After 10 min, a water ice mixture (1,000 mL) was added for solvent exchange for 24 h. The spheres were sieved and washed with an excessive amount of distilled water six times to remove glycerol residue. The spheres were then lyophilized for 3 days.

Synthesis of PLLA-PEG-PNIPAm random copolymer: Similar to the synthesis of HEMA-PLLA macromonomer, PEGMA-PLLA10 macromonomer was first synthesized as follows: L-lactide (40 mmol, 5.760 g), PEGMA (4 mmol, 2.0 g) and $Sn(Oct)_2$ (0.4 mmol, 0.162 g) were mixed in a 50 mL round-bottom flask with stirring and nitrogen purging. The mixture was heated to 120℃ under nitrogen protection for complete melting. The polymerization was carried out at 140℃ for 2 h. The crude product was dissolved in 20 mL chloroform, precipitated in 100 mL cold methanol, and then vacuum dried. PLLA-PEG-PNIPAm random copolymer was synthesized as follows: PEGMA-PLLA10 macromonomer (1.4 g), PEGMA (2 mmol, 0.1 g), NIPAm (12.4 mmol, 1.4 g) and AIBN (0.06 mmol, 9.8 mg) were added into dioxane (10 mL) and were stirred until dissolved. The polymerization was carried out at 70℃ for 24 h. After polymerization, the crude product was purified by repeated re-precipitations from chloroform to methanol for 3 times, and finally vacuum dried at 40℃ for 48 h. Measured by GPC and calculated using polystyrene as standards and THF as eluent: Mn=16630, Mw=24114; Calculated from the average signal intensity ratios of methylene protons of PEG, methine protons of PNIPAm, and methine protons of PLLA, the mass ratio of PLLA, PEG, PNIPAm in the copolymer: 52/14/34wt%.

NMR measurements: ^1H spectra of the macromonomers and copolymers were recorded with an Inova 400 NMR instrument operating at 400 MHz at room temperature using $CDCl_3$ as the solvent.

Molecular weight measurement: The molecular weight was measured using a Waters gel permeation chromatograph (GPC) model 440. THF was used as the mobile phase at a flow rate of 1.0 mL/min. Molecular weight and polydispersity of macromonomers and copolymers were calibrated with polystyrene standards.

Dynamic light scattering (DLS) measurements. The hydrodynamic diameter of polymers was evaluated using a Zetasizer Nano ZS dynamic light scattering (DLS) instrument (Malvern, UK). A wavelength of 633 nm and a scattering angle of 173° were fixed. The dispersant refractive index and the viscosity of water were set to be 1.330 and 0.8872 cP, respectively. The measurements were performed from 25-50℃.

Confocal imaging: Various microspheres were treated with 10% w/v rhodamine-conjugated BSA aqueous solution for 20 min, followed with extensive washing using deionized water. The microspheres were examined using confocal laser scanning microscopy (CLSM) (Nikon Eclipse C1).

Scanning electron microscopy (SEM) observation: The surface morphology of the copolymer

microspheres was examined using SEM (Philips XL30 FEG) with an accelerating voltage of 8 kV. The samples were coated with gold for 90 s using a sputter coater (DeskII, Denton vacuum Inc). During the coating, the gas pressure was kept at 50 mtorr and the current was 18 mA.

Fiber diameter determination: The average fiber diameter was calculated from the SEM micrographs. At least 100 fibers were measured for each sample, and their averages and standard deviations were reported.

***In vitro* degradation of NF-GMS**: NF-GMS (100 mg) were immersed in phosphate buffer solution (10 mL, 0.1 M, pH 7.4) on an orbital shaker at 37°C with a shaking speed of 50 rpm. The buffer solution was renewed every other day. At preset time intervals, the samples were removed from the buffer solution and dried to constant weights under vacuum at room temperature. The morphological changes were examined using SEM.

NF-GMS size distribution: The size distribution of PLLA-PEG-PNIPAm microspheres (60-90 μm) was measured using a Multisizer 4 Coulter Counter (Beckman Coulter, Inc., Fullerton, CA) equipped with 140 μm and 1000 μm apertures for different size analysis. The microspheres were dispersed in 0.9% saline for measurement. The size distribution was first measured with a 140 μm aperture to analyze the size distribution in the range of 2.8 μm to 80 μm. Then, a 1000 μm aperture was used to analyze 20 μm to 600 μm distribution. These two measurements were merged by the Multisizer 4 software.

Rheological test: The rheological properties of the hydrogels were monitored using an AR2000 Rheometer (TA instruments, United States) equipped with a temperature controller. Parallel plates with 20 mm diameter were used for all the tests. The gap distance between the plates was 0.4 mm. For measurements other than frequency spectrum, a constant 1 rad/s angular speed was used. For measurements other than stress sweeps, a constant 0.1 Pa stress was used. Temperature sweeps were performed on samples from 20°C to 45°C with a heating rate of 2°C/min. Frequency sweeps ranging from 0.1 to 100 rad/s were conducted at 37°C. Stress sweeps were performed on samples from 1 to 1000 Pa at 37°C.

Cell survival assay of co-cultured CMs and NF-GMS

5 million CMs mixed with Collagen-I or NF-GMS at the ratio of 30:1 were co-cultured in tube with CDM3 medium for 2 h to form the cell pellets. Then the cell pellets were treated with 50 μM H_2O_2 for 4 h to mimic in vivo MI injury. Proteins were extracted in lysis buffer followed by centrifugation at 4°C for 15 minutes at 12,000 rpm. Protein concentration was measured by Bradford protein assay and 40 μg of total protein was separated by SDS-PAGE and then transferred to PVDF membranes. The membranes were blocked with 5% nonfat dry milk for 1 h at room temperature and then incubated with primary antibodies against Bax and Bcl-2 (key markers of cell apoptosis) overnight at 4°C. After 3 washings with TBST, the membranes were incubated with secondary antibody in TBST solution for 1 hour at room temperature. After 3 washings, the

membranes were scanned and quantified by Odyssey CLx Imaging System (LI-COR Biosciences, USA).

Histology and Immunohistochemistry

Hearts were fixed in 4% paraformaldehyde, frozen in Tissue-Plus O.C.T Compound (Fisher Scientific), and cryosectioned into 7 μm sections for immunohistochemistry and histological analyses. Slide sections were permeabilized with 0.3% Triton X-100 for 15 min at room temperature, blocked with 5% horse serum in DPBS-T for 1 h at room temperature and incubated with primary antibodies against human mitochondria (HuMito), Cnnx43, CD68, CD 31 and αSMA at 4°C overnight in 2% horse serum. Sections were then washed 3 times with PBS for 15 min each time, incubated with Alexa Fluor secondary antibodies in 2% horse serum in DPBS-T for 1 h at room temperature, washed with PBS for 3 times and 15 min each time, then stained with DAPI, and images were obtained by a Nikon A1 Confocal Laser Microscope.

References

[1] a) D. Roy, W. L. A. Brooks, B. S. Sumerlin, *Chemical Society Reviews* **2013**, 42, 7214; b) L. Mi, H. Xue, Y. Li, S. Jiang, *Advanced Functional Materials* **2011**, 21, 4028.