Supporting Information

Biohybrid methacrylated gelatin/ polyacrylamide hydrogel for cartilage repair

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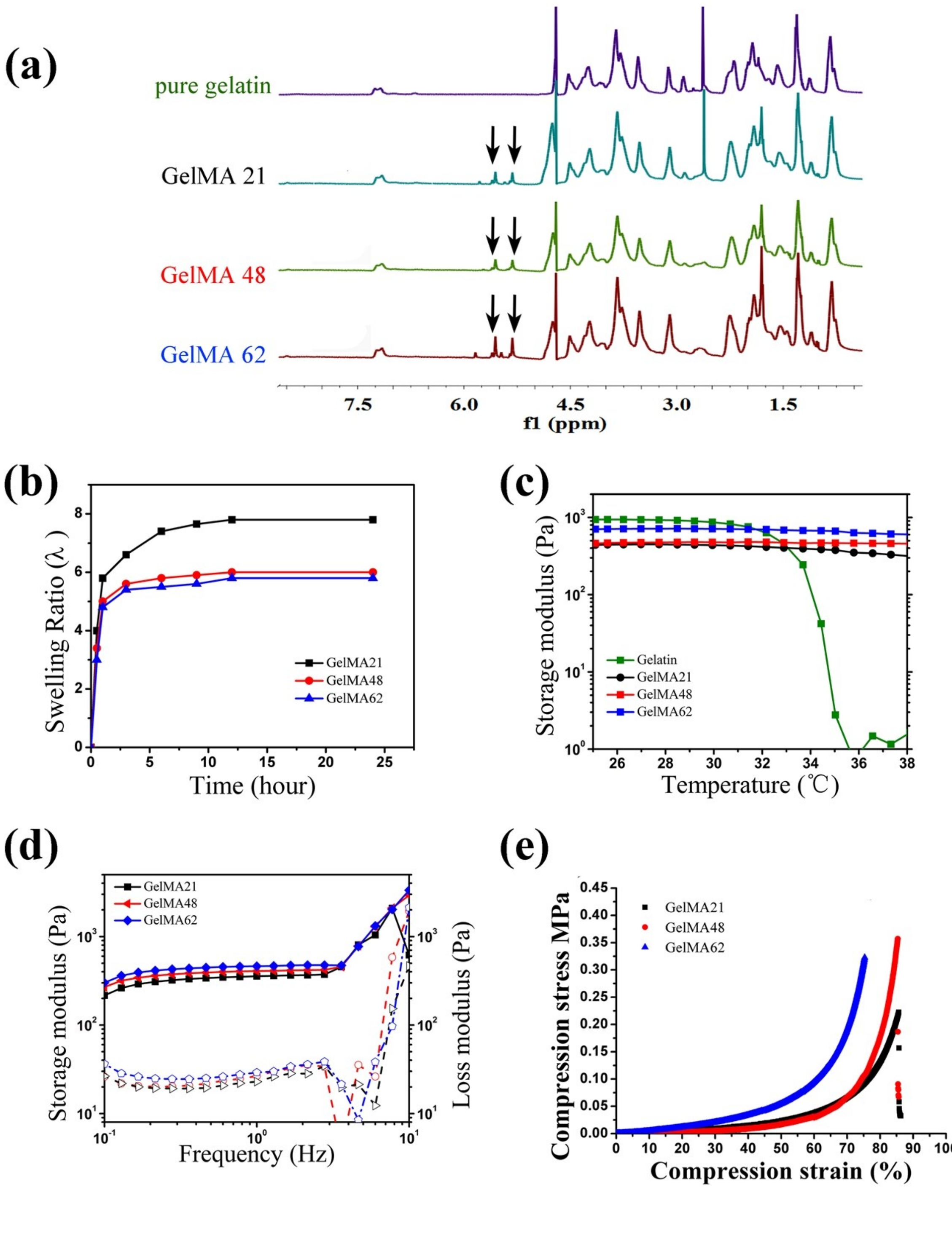

Figure S1. (a) ^{1}H-NMR spectra of GelMA. (b) Swelling behaviors of GelMA hydrogels. (c) Thermal scanning rheological test of G' for GelMA hydrogels. (d) Frequency-scanning rheological test of GelMA hydrogels. (e) Compression stress-strain curves of GelMA hydrogels.

Experiments:

Before preparing GelMA/PAM hydrogel, we first synthesized three kinds of GelMA with meth-acrylamide (MA) degree of 21%, 48%, and 62%. Then, we fabricated three kinds of pure GelMA hydrogels using GelMA with different MA degrees by UV light-induced polymerization. The hydrogels were denoted as GelMA 21, GelMA 48, GelMA 62. The thermal stability, viscoelasticity, compression strength, and swell ratio of these hydrogels were tested so as to screen the optimal MA degree.

Results:

The success of MA modification of gelatin and the MA degree of GelMA was determined by ^{1}H NMR spectrum, as shown in Fig. S1a. Compared with the ^{1}H-NMR of gelatin, two protons at δ = 5.64 ppm and δ=5.36 were observed in the ^{1}H-NMR of GelMA, as pointed by black arrows in Fig. S1a. These peaks were belong to the $-H_2C = C(CH_2)-$, indicating the success of MA modification. The MA degree of GelMA was calculated as 100% X/Y, in which X was the integral of the protons at the double bond, and Y was the integral of anomeric proton at δ = 4.75 ppm. According the assignment of the ^{1}H-NMR spectra in Fig. S1a, the MA of GelMA was 21%, 48%, and 62%.

The swelling tests showed that GelMA 48 and GelMA 62 hydrogels had smaller swell ratio than GelMA 21 hydrogels (Fig. S1b). Figure S1c is the thermal scanning rheological observation for the elastic modulus of GelMA hydrogels and gelatin hydrogel, which indicated that the GelMA hydrogels have good temperature stability even the temperature is higher than 37 degree. This result suggested that the GelMA hydrogel was able to maintain their shape and mechanical properties even at body temperature. The frequency sweeping rheological tests showed that both the elastic and viscous modulus of GelMA hydrogels increased with the increase of MA degree (Fig. S1d). The compression tests showed that the compressive strength also increased with MA degree. However, excessive high MA degree (62%) led to the decrease of the compressive strength, which is because high MA degree results in high crosslinking density and consequent high brittleness of the GelMA hydrogels (Fig. S1e). GelMA 48 has the highest compressive strength among all the samples. Thus, GelMA with MA degree of 48%

was used to prepare GelMA/PAM hydrogel in our study.

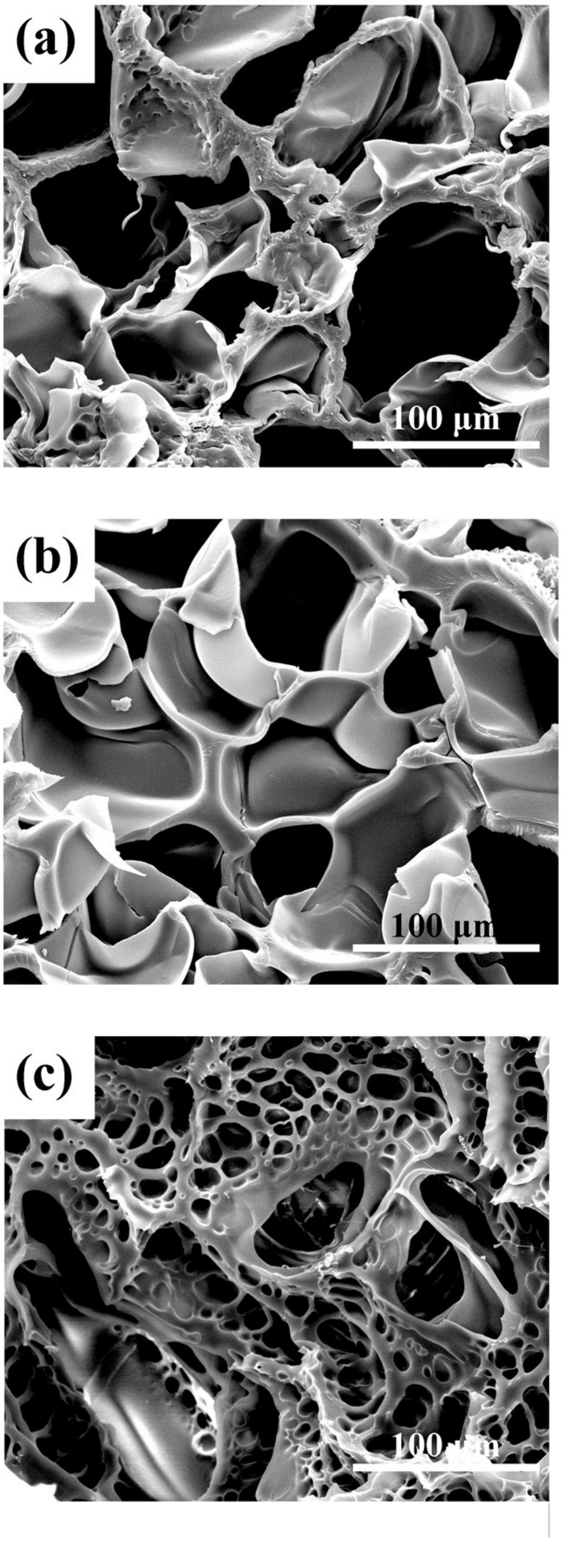

Figure S2. SEM micrographs of GelMA/PAM biohybrid hydrogel with different GelMA/AM mass ratios. (a) GelMA: AM = 1 : 0.25, (b) GelMA: AM = 1 : 0.5, (c) GelMA: AM = 1 : 1.

Table S1 Primers used for qRT-PCR.

Gene		Sequence (5'-3')	Amplicon size (bp)
Aggrecan	forward	GCCACTGTTACCGTCACTTC	136
	reverse	TAGTCCTGAGCGTTGTTGTTG	
GAPDH	forward	TTCCACGGCACGGTCAAG	116
	reverse	TACTCGGCACCAGCATCAC	

Table S2. ICRS gross observation of cartilage repair.

Cartilage repair assessment ICRS		Points
Degree of defect repair	In level with surrounding cartilage	4
	75% repair of defect depth	3
	50% repair of defect depth	2
	25% repair of defect depth	1
	0% repair of defect depth	0
Integration to border zone	Complete integration with surrounding cartilage	4
	Demarcating border <1mm	3
	3/4 of graft integrated with surrounding	2
	With a notable border >1mm width and 1/2 of graft integrated with surrounding	1
	From no contact to 1/4 of graft integrated with surrounding cartilage	0
Macroscopic appearance	Intact smooth surface	4
	Fibrillated surface	3
	Small, scattered fissures or cracks	2
	Several, small or few but large fissures	1
	Total degeneration of grafted area	0
Overall repair assessment	Grade I: normal	12
	Grade II: nearly normal	8-11
	Grade III: abnormal	4-7
	Grade IV: severely abnormal	0-3

Table S3. Modified O'Driscoll Scoring histological evaluation of cartilage repair.

Features		Points
Staining	Normal	4
	Slight reduction	3
	Moderate reduction	2
	Severe reduction	1
	No dye noted	0
Integrity of surface	Surface smooth and intact	5
	Surface horizontal fibrillation	4
	Surface shows fissures to 25–100% of the depth of the cartilage	3
	Interruption	2
	Serious deep interruption of the surface and many deep fibrillations	1
Cell distribution	Columnar	3
	Mixed/columnar-clusters	2
	Clusters	1
Thickness	100% of normal host cartilage	5
	75-100% of normal host cartilage	4
	50-75% of normal host cartilage	3
	25-50% of normal host cartilage	2
	25-0% of normal host cartilage	1
Subchondral bone	Normal	3
	Increased remodeling	2
	Bone necrosis/granulation tissue	1
	Detached/fracture/callus at base	0
Architecture surface	Normal	4
	Slight fibrillation or irregularity	3

	Moderate fibrillation or irregularity	2
	Severe fibrillation or irregularity	1
Surface of area filled with cells	100-90%	5
	90-75%	4
	75-50%	3
	50-25%	2
	25-0%	1
Bonding to adjacent cartilage	Bonded at both ends	3
	Partially at both ends	2
	No bonded	0
Basal integration	100-90%	4
	90-70%	3
	70-50%	2
	50-0%	1
Inflammation	No	5
	Slight	3
	Severe	1